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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Twenty-ninth Meeting of The Nutrition Society was held at the Royal Society of Arts, John Adam Street, London, W.C.2, on Friday, 4 December 1959, at 10.30 a.m., when the following papers were read:

The effects of intraruminal infusions of acetic, propionic and butyric acids on nitrogen retention in growing heifers. By J. A. F. Rook, C. C. Balch and R. C. Campling, National Institute for Research in Dairying, Shinfield, Reading

Armstrong & Blaxter (1957) showed, with fattening sheep, that for lipogenesis the efficiency of utilization of acetic acid is markedly less than that of propionic and butyric acids. In growth, however, protein deposition is of equal or greater importance than fat deposition. As part of a wider investigation we have studied in growing heifers the effects on nitrogen retention of adding individual volatile fatty acids to a basal ration.

Four Friesian heifers, approximately 2 years of age and 850 lb in live weight, each received daily a basal ration of 5.4 kg hay, 1.1 kg cubed dairy concentrate and 227 g decorticated groundnut cake and 10 gal of water as a slow, intraruminal infusion. Treatments were water alone, as control, or 3500 kcal as either acetic, propionic or butyric acids added to the water. The design was a 4×4 balanced Latin square, with periods of 35 days. Determinations of nitrogen retention and dry-matter digestibility were made on days 13-24, of 'empty body' weight (live weight after the temporary removal of the contents of the reticulo-rumen) on days 4 and 25, and of the consumption of hay offered ad lib. on days 26-35.

Treatment mean values were:

	Nitrogen	Change in	Dry-matter	Ad lib.
	retention	'empty body'	digestibility	hay intake
Treatment	(g/day)	weight (lb/day)	(%)	(lb/day)
Control	14.6	-0.24	56.2	16.6
Acetic acid	22.0	+0.59	56.2	14.4
Propionic acid	18.2	+0.38	57.2	15.4
Butyric acid	19.8	+0.75	56.9	15.6
Standard error of difference	± 3°24	±0·385	± 0.97	± 0·56

Though supplementary acetic acid affected appetite, digestibility of dry matter was not affected by any treatment and the observed effects on nitrogen retention and 'empty body' weight should reflect the direct effects of the supplements. It appears that, in heifers growing relatively slowly, rations which produce a rumen fermentation in which acetate production is high in relation to that of propionate may offer the most efficient utilization of feed energy.

REFERENCE

Armstrong, D. G. & Blaxter, K. L. (1957). Brit. J. Nutr. 11, 413.

Metabolic studies on isocaloric submaintenance diets, over a period of weeks. By Margaret Carey, K. Hodd, Henrietta Koerselman, T. Pilkington, V. M. Rosenoer, F. Mary Simmonds and A. Otaki, St. George's Hospital, London, S.W.1

Studies have been carried out in our metabolic unit with very co-operative patients for periods of several weeks.

Kekwick & Pawan (1953, 1956) stated that weight loss over short periods on isocaloric submaintenance diets was greater when most of the calories were supplied by fat than when most were supplied by carbohydrate.

We have found that on 800 and 1000 kcal diets very obese patients lose weight at a steady rate over periods of up to 4 months.

There is no significant difference in the rates of weight loss when isocaloric submaintenance diets consisting mainly of fat or mainly of carbohydrate are compared if the periods of study are long enough to achieve a 'steady state'.

When these diets are interchanged, deviations from the weight curve occur lasting up to 10 days. These can be accounted for mainly by changes in the fluid balance.

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A microbiological method for assessing the nutritional value of proteins.

By J. E. Ford, National Institute for Research in Dairying, Shinfield, Reading

Streptococcus zymogenes needs broadly the same exogenous amino acids as the growing rat. It is powerfully proteolytic and grows quickly with an adequate intact protein as the main source of nitrogen. I have developed a method of assay in which a strain of S. zymogenes is used to provide an estimation of 'protein quality', and have measured the relative nutritional values of seventeen protein foodstuffs for which rat-assay values had been determined by Dr K. M. Henry. The results obtained by the two methods were closely correlated, the coefficient of correlation being o.g. (P < 0.001). Beneficial effects for S. zymogenes of amino-acid supplementation of certain proteins have been demonstrated. Wheat gluten, relative to the protein of whole egg (=100), is deficient in methionine, lysine and isoleucine. Making good the deficiency of methionine by addition of 2 g L-methionine/16 g nitrogen increased the relative nutritive value from 54 to 68. The corresponding addition of 4 g L-lysine increased the latter value to 85, and the further addition of 3 g L-isoleucine gave a value of 94. Similarly, the addition of methionine (1% w/w) increased the relative nutritive value of soya-bean meal from 74 to 94, and of roller-dried skim milk from 93 to 109.

The technique should offer a relatively simple means for studying supplementary relationships between proteins, and the effects of heat treatment and processing on nutritive value. It is potentially useful for measuring certain individual amino acids

—methionine, tryptophan, arginine, histidine, leucine, isoleucine, valine and glutamic acid—in intact proteins.

Vitamins A and E in human blood. 1. Levels of vitamin A and carotenoids in British men and women 1948-57. By Z. A. Leitner, 52 Welbeck Street, London, W.I., T. Moore and I. M. Sharman, Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council

Vitamin A and total carotenoids were estimated during the decade 1948-57 in the blood of over 700 British men and 500 women in whom no serious disease could be detected. Wide individual variations were found in both sexes. The averages, for equal numbers of each sex, were 158 i.u. of vitamin A and 126 µg of carotenoids per 100 ml of serum. In groups of men, whether arranged according to age, season or biennial period, averages for vitamin A were always some 20% higher than for women. For carotenoids this relationship was reversed, with averages about 8% higher for women than for men. The difference in vitamin A between men and women tended to widen with increasing carotenoid levels. In men vitamin A averages increased with age until a maximum in the age group 50-59 years was reached. In women vitamin A also increased with age until at 70-79 years the usual sex difference had disappeared. Carotenoids in men went almost parallel to vitamin A, but in women unexpectedly high values were found under the age of 30 years. In both sexes higher averages for carotenoids were found in spring and summer than in autumn and winter. In women vitamin A varied little between seasons but in men the averages in summer and autumn were slightly higher than in winter and spring. In both sexes blood vitamin A appears to have increased since the removal of wartime food restrictions. The increases appear to have run roughly parallel with increases in the intake of vitamin A, as estimated in the National Food Survey. Increases in blood carotenoids over the same periods were less pronounced. It seems probable that the sex differences in the levels of vitamin A and carotenoids may be due, at least in part, to a greater efficiency in the conversion of carotene in men than in women. Some of our results have already been reported (Leitner, Moore & Sharman, 1952).

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The influence of a meat diet on the retention of nitrogen, phosphorus and calcium in growing kittens. By A. Heulwen Roberts and Patricia P. Scott, Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.1

Litter-mate weaned kittens were maintained in individual metabolism cages and balances carried out over 8- (occasionally 4-) day periods to determine the total gain or loss of nitrogen, phosphorus and calcium on different diets.

On stock diet (high protein, high carbohydrate, adequate mineral elements) retentions followed a consistent pattern, were positive and were related to the overall weight gain in the period under study. Smaller, but still positive retentions of all three elements occurred even when there was no increase in body-weight during the balance period.

On unsupplemented heart (high protein, high fat, low Ca, Ca:P \sim 1:20) the pattern of retention was quite different. At first N and P retentions were high, while Ca was just positive or slightly negative. After several weeks on raw heart, the length of time depending on the initial age, weight and state of health of the kittens, N and P retentions fell markedly, though they remained slightly positive even when the animals were losing weight. Ca balances at this stage were negative, loss of Ca occurring in both urine and faeces. Supplementation of raw heart with Ca (0.5 g/100 g wet diet, Ca:P \sim 1:1) after a period on the unsupplemented diet usually resulted in an immediate positive Ca balance, accompanied by a marked increase in N and P retentions and overall weight gain. But some kittens, especially those showing infections or the dystrophic signs previously described (Greaves, Scott & Scott, 1958, 1959) took some time (1-2 weeks) to get into positive Ca balance once more. This may account for the persistence of signs in clinical cases of calcium deficiency under treatment.

After several weeks on raw heart, when kittens were in marked negative Ca balance, a supplement of 100 μ g iodine/cat/day without additional Ca effected a marked improvement converting the Ca loss into a slight positive retention. This agreed with the clinical improvement noted previously on treatment with supplementary iodine.

When Ca and I supplements were given together, good growth and retention were observed on raw heart, but the overall pattern of retention still differed from that on the stock diet.

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A species difference in the response of the thyroid to the addition of calcium and iodine to a meat diet. By Patricia P. Scott, Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.1, and M. G. Scott, Histology Division, Royal Veterinary College, London, N.W.1

Hyperaemia, hypertrophy and hyperplasia of the thyroid gland were observed in weaned kittens that had been fed on raw heart alone (Greaves, Scott & Scott, 1958). The condition was progressive, severity depending on the length of time on the diet; after 30 weeks little colloid remained and the thyroids began to show atrophic, fibrotic change in place of hyperplasia. Estimations carried out for us by the Chilean Iodine Bureau showed that the diet provided 5-40 µg iodine/kitten/day.

When sufficient calcium gluconate or carbonate was added to raw heart to bring the Ca:P ratio to 1.0, or when I was given at the rate of 100 μ gI/kitten/day, the

glands were pale brown in colour, lighter (Table 1) and contained some colloid. When the supplements of Ca and I were given together, the glands were lighter still and histologically normal, that is similar to those from animals of the same age fed on stock diet.

Since these observations conflicted with the findings of workers such as Thompson (1933) who stated that Ca is goitrogenic for rats, we repeated our experiments using

Table 1. Mean weights of thyroids (with parathyroids) expressed as mg/100 g bodyweight on raw-heart diet

	Alone	+Ca	$+\mathbf{I}$	+Ca+I
Kittens (8 weeks	17·61 ± 0·99	11.32 ± 1.51	10.49	8.52
or longer on diet)	(9)	(10)	(4)	(2)
Young ♂ rats	14.72 ± 0.74	17.85 \pm 0.67	6·38 ± o·36	6.23 ± 0.17
(6 weeks on diet)	(10)	(10)	(9)	(10)

Figures in parentheses are the number of animals in each group.

weanling male rats. The rats ate the diet well but by 4 weeks the unsupplemented group showed similar signs to the kittens. At autopsy after 6 weeks severe osteoporosis was apparent without any sign of rickets, while the thyroids were hyperaemic, hypertrophied and hyperplastic. The rats receiving additional $CaCO_3$ at the same rate as the kittens had even heavier (Table 1) thyroids than the unsupplemented group. Supplementation with I at the rate of 8.5 μ g/rat/day resulted in histologically normal thyroids of similar weight to those of a control group on modified diet 41B. When both Ca and I were added to the heart diet weights of the thyroid were similar to those of the group supplemented with I alone, but hyperplasia was apparent on histological examination.

These observations show that cats and rats respond differently to supplementation of a meat diet with Ca at physiological levels.

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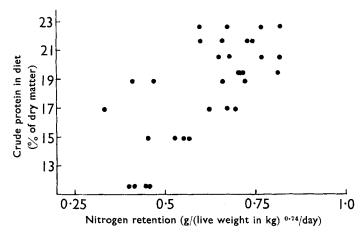
The effect of varying levels of dietary protein on nitrogen retention in early-weaned calves. By T. R. Preston, F. G. Whitelaw*, R. D. Ndumbe and Euphemia B. Charleson, Rowett Research Institute, Bucksburn, Aberdeen

Four diets of approximately the same energy value and containing respectively 14.9, 16.9, 19.4 and 21.6% crude protein in dry matter were each fed in turn to four 11-week-old Ayrshire bull calves according to a Latin square design. The calves had been weaned from whole milk on to a dry concentrate at 3 weeks old as in the rearing system described by Preston (1956). The basal diet contained (parts by weight) ground flaked maize 40, ground oats 20.75, grass meal 10, molassine meal 15,

^{*}In receipt of an Agricultural Research Council Scholarship.

extracted decorticated groundnut meal 22·5, minerals 1·5, vitamins A and D supplement 0·5, Aurofac 2A 0·25; the other diets were formulated by partial replacement of the groundnut meal with ground barley. Each diet was fed for 10 days and measurements for nitrogen balance were made over the last 5. Feeding was at the daily rate of $\frac{8}{100}$ (live weight in kg)0·74 based on the weight of the calf at the beginning of each 10-day period.

The above procedure was repeated with a second group of four calves and diets containing protein levels of 11.6, 18.8, 20.5 and 22.6%.



The results shown in the figure indicate that nitrogen retention increases to a maximum as the crude protein in the dry matter of the diet approaches 20% and then levels off.

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Starch refection: a misnomer. By T. B. Morgan, Department of Nutrition, Queen Elizabeth College, University of London

Starch refection was first observed by Fridericia (1926) in rats fed diets containing rice starch. All of the workers who concerned themselves with this problem, e.g. Kon, Kon & Mattick (1938), Hopkins & Leader (1945–6) and Fridericia himself, have held that the phenomenon was due to vitamins being synthesized by intestinal microflora and subsequently absorbed from the intestine. This occurred because the starches producing refection were poorly digested; some therefore reached the caecum and increased microbial growth and vitamin synthesis. Refection and coprophagy were considered to be two separate phenomena by all workers. Although Roscoe (1931) thought that coprophagy was necessary for refection to occur, the effect was thought not to be due to re-ingestion of vitamin B since the faeces were said not to contain any.

With the technique of preventing coprophagy devised by Barnes, Fiala, McGehee & Brown (1957) I have now been able to show that starch refection occurs only when

rats eat their faeces. Since there is now little evidence that vitamins synthesized in the gut of experimental animals can be absorbed when coprophagy is not practised, it is suggested that the term refection should be abandoned. There are three reasons for this. Firstly, those who have worked on the phenomenon have assumed that it involved direct intestinal absorption of the vitamins as well as their synthesis. Secondly, in spite of this, many other workers have read into the word 're-fection' the ideas of 're-ingestion' and 'faeces'. They have therefore confused refection and coprophagy. Thirdly, the word refection has been used for at least 600 years to mean simply the partaking of refreshment and not in the restrictive way it has been used by nutritionists.

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The food intake and energy expenditure of some elderly women living alone. By J. V. G. A. Durnin, Elaine C. Blake, Margaret K. Allan and Elizabeth J. Shaw, *Institute of Physiology, University of Glasgow*

Some evidence that a relative dietary excess of carbohydrate is an important aetiological factor in kwashiorkor. By I. Macdonald, *Physiology Department*, Guy's Hospital Medical School, London, S.E.1

Large numbers of people in tropical regions live on a diet which contains a relative excess of carbohydrate with, of course, numerous other deficiencies, notably protein. The malnutritional disorder, called kwashiorkor, found in children residing in the tropics, is associated with a diet having such a relative excess of carbohydrate. The diet of these children is deficient in protein but a protein deficiency not associated with a carbohydrate excess gives rise to a different clinical picture (marasmus) and it is possible that the differences found between kwashiorkor and marasmus may be due to the relative carbohydrate excess in kwashiorkor.

To support the view that a relative excess of carbohydrate in the diet contributes to pathological changes the following evidence is presented:

(1) Daily electrophoresis of serum proteins in children with kwashiorkor and with marasmus for the 1st fortnight of similar treatment showed no difference in the pattern of recovery of the serum proteins, except in degree. Furthermore the proportional fall found at the commencement of treatment in all the fractions of the serum proteins, except albumin, was similar in kwashiorkor and marasmus, and was characterized by a grouping of α_1 - and γ -globulins and α_2 - and β -globulins. This similar protein pattern in both types of malnutrition suggests that pathological difference found between them may not be due to protein.

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(2) The fat in the classical fatty liver of kwashiorkor differs in composition from that found in other diseases (e.g. chronic infection) associated with a fatty liver. The principal differences in composition consist of a normal proportion of non-saponifiable fats in the fatty liver of kwashiorkor, whereas in the other fatty livers this fraction rises. Similarly there is no rise in the phospholipid fraction in the liver fat of kwashiorkor.

These differences in composition are associated with a diet that has a relative excess of carbohydrate and are the kind of change that might be expected if excess carbohydrate was converted into fat in the liver and not transported. In marasmus the proportion of liver fat is normal.

(3) Experimental animals fed on a diet containing an excess of readily available carbohydrate show fatty changes in the liver, and an increase in hepatic reticulin.

The central nervous system of pigs on low-protein diets. By B. S. Platt and R. J. C. Stewart, Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7

There is evidence from clinical observations that the central nervous system (C.N.S.) is affected in protein malnutrition and it has been suggested that, in the florid form, human and animal subjects die a 'C.N.S. death' (Platt, 1959, 1960). If this proves to be so and the mechanism is elucidated, rational treatment may be possible of acute malnutrition in infants and children among whom mortality is now very high (Garrow, 1959; Waterlow, 1960). There are no studies reported of the neuropathology of protein malnutrition (cf. Meyer, 1958; Trowell, Davies & Dean, 1954), although the effect of experimental inanition has been described (see, for example, Andrew, 1941).

Pigs were fed on normal, low-protein (LP) and low-protein plus carbohydrate (LP+CH) diets (Heard, Platt & Stewart, 1958). Others received diets (5CLP and 20CLP) in which casein (5 or 20% w/w) replaced an equal weight of starch in the LP diet. LP and CLP diets were fed at isocaloric levels.

Appearance of nerve cells of the spinal cords of protein-malnourished and normal pigs

Diet	No. of animals	Quantity* of Nissl substance	Nissl granules	Foaming	No. of glial nuclei	Satellitosis		
LP+CH	7	Reduced	Dust-like	Marked	Increased	Marked		
LP	9	Reduced	Dust-like	Present	Increased	Present		
5CLP	2	Slightly reduced	Fair to good	Absent	Slightly increased	Absent		
Normal	5	Good	Good	Absent	Normal	Absent		
*As judged by the Einarson (1932) staining technique.								

'Foaming' resembles, but is less intense than, the ring formation seen in Nissl's severe cell change. Satellitosis, probably due to oligodendroglia, is reported when glial nuclei are clustered around an abnormal cell.

When LP animals are fed on the 20CLP diet, nerve-cell recovery is slow; after 1 month the satellitosis appears to be more active, although the amount of Nissl substance is increased; even after 3 months the cells are not normal.

Changes similar to those tabulated have been seen by us in the higher levels of the C.N.S. of malnourished animals and also in a specimen of brain stem obtained for us by Professor R. N. Chaudhuri from a case of kwashiorkor in a child.

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Electroencephalographic and electrocardiographic technique in nutritional studies on laboratory animals. By G. Pampiglione, R. J. C. Stewart and B. S. Platt, Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7

In acute experiments a large variety of techniques have been described for recording the electrical activity of the brain (E.E.G.) and of the heart (E.C.G.). In experiments lasting weeks or months, however, particularly in growing animals, most of the techniques described in the literature are unsuitable or cumbersome. A simple method has been devised for study of E.E.G. in growing animals which is also applicable to nutritional studies, particularly in dogs and pigs. Implanted electrodes (either individually or on a plate) and 'stick on' electrodes have disadvantages which were overcome by using as electrodes small stainless steel needles (Lane's cleft palate needles, 3/8 in.) soldered to an extremely flexible lead ('deaf aid' wire). The needles can be retained in position by insertion into the skin, thereby avoiding penetration into the muscles, which is painful and stimulates muscle activity. Collodion may be added to secure the electrode to the skin but in practice this was found to be unnecessary. Half a dozen electrodes may be appropriately placed on the scalp in $\frac{1}{2}$ min and two or more electrodes can be similarly attached to the skin of the legs to record the E.C.G. A surface anaesthetic used in dentistry (Xylotox paste) was useful in a few animals with a sensitive skin. The young pups were gently held on the lap of a laboratory assistant; the pigs were suspended in a sling. The technique has been successfully employed for nearly 2 years.

Illustrative records will be presented of the alterations that may occur in the E.E.G. and the E.C.G. of thiamine-deficient growing pups before and after administration of thiamine.

This work was in part supported by a grant from the National Spastics Society Medical Research Fund.

Pathological changes in the pars anterior of the hypophysis of animals maintained on low-protein diets. By K. O. Godwin and B. S. Platt, Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, Mill Hill, London, N.W.7

Jackson (1925) described atrophic changes in the pituitary gland of rats in chronic inanition. Mulinos & Pomerantz (1940) used the term pseudohypophysectomy to describe the response of the endocrine organs of the rat to chronic malnutrition 'not only because of the resemblance between the effects of inanition and hypophysectomy but also because [they believed] that many of the effects of inanition are due to malnutrition of the hypophysis, resulting in a diminished secretion of hormones'. Pathological changes in the pituitary gland have been reported in rats on diets deficient in different single amino acids; the first report in a series by Scott and his colleagues is by Schwartz, Scott & Ferguson (1951).

The pathology of the pituitary gland has been studied (Tejada & Russfield, 1957) in a series of Guatemalan children suffering from malnutrition. All except one, however, died with intercurrent infection. Other work has been mainly on adults suffering from malnutrition (Zubirán & Gómez-Mont, 1953) and anorexia nervosa (Wilson, 1954).

The pituitary glands from twenty-five pigs have been examined; five controls on a commercial diet, six on low-protein (5%), four on low-protein supplemented with 5% casein, six on low-protein with additional carbohydrate (see Heard, Platt & Stewart, 1958) and five animals were recovering from protein malnutrition.

The pathological changes in the pars anterior include: (1) the structural features of the pars anterior as seen under low magnification (\times 100) are difficult and sometimes impossible to recognize; (2) an increase in the number of cells per field (6 μ sections), accompanied by crowding of nuclei and loss of cytoplasm of cells; (3) in some portions of the gland where there is still demonstrable cytoplasm and the three types of cells can still be differentiated, there is vacuolation; (4) in other portions the cytoplasm is so much reduced that its presence cannot be demonstrated by the usual staining techniques; (5) some of the nuclei are 'hyperchromatic' and show evidence of necrosis; (6) small cysts are found throughout the pars anterior.

Changes (1)–(5) are reversible as they can be shown to regress during recovery of the animal from protein malnutrition.

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Effect of protein value of the diet on the course of malarial infection (Plasmodium berghei) in rats. By B. S. PLATT, I. S. DEMA and D. S. MILLER, Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7

Rats infected with Plasmodium berghei show some changes similar to, but more marked than, those encountered in protein malnutrition in uninfected animals

Summary* of observations on P. berghei infection of rats on diets of differing protein value

Group	N.D-p.V.† of diets (g/100g diet)	No. of	Peak para- sitaemia‡	Mortality (%)	Weight of spleen (g/100g rat)	Weight o liver (g/100g rat)	Haemoglobin concentration (g/100 ml blood)	Serum p Total (g/100 ml)	roteins Ratio, albumin: globulin
Infected	3.4; 4.3	35	43	4	1.6	5.7	11.0	3.9	1.0
	7.8; 8.0; 8.3	72	87	43	2-4	7.1	4.4	4.1	1.0
Pair-fed	3.4: 4.3	24	_	0	0.7	5-1	13.1	4.7	1.0
controls	7.8; 8.0; 8.3	44	_	0	0.8	5.6	15.3	6.3	0.9

(Dema, Miller & Platt, 1959). In this communication differences are summarized between animals on diets of different protein values inoculated with equal amounts of blood from a malarious rat.

The malarial infection in rats is more severe in those on diets of high than of low protein value. These results are of interest in view of the observations of Ramakrishnan (1954a) on the effects on the course of parasitaemia in rats of periods of starvation followed by normal feeding. It is difficult, however, to relate our results to those of the same author (Ramakrishnan, 1954b) without quantitative data on the protein values of the diets he used.

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The water-soluble vitamin content of blood serum and milk of the bitch.

By D. J. NAISMITH and P. L. PELLETT, Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7

Although the dog from birth does not require a dietary source of ascorbic acid, the vitamin is concentrated by the mammary gland (Naismith, 1958); the ratio of milk ascorbic acid:blood-serum ascorbic acid was found to be approximately 4:1.

In attempting to explain this apparent paradox, the possibility was considered that other water-soluble vitamins might be concentrated to the same extent. Since

^{*}For fuller information see Dema (1959). †See Platt & Miller (1959). ‡Mean percentage of erythrocytes parasitized between the 5th and 7th day after infection.

	Blood serum			Milk (defatted)				
Vitamin Ascorbic acid (µg/ml)	Mean value 7·2	No. of observations 3 (3)	Range 4·6-8·8	Mean value 31.9	No. of observations 3 (3)	Range 17·8–46·0		
Biotin (mµg/ml)	3.2	9 (6)	1.7–5.8	95.0	5 (5)	68.0-134.0		
Cyanocobalamin* (mµg/ml)	0.02	5 (4)	0.03-0.02	8.3	5 (4)	1.6–22.0		
Folic acid (mµg/ml)	Not determined			2.2	2 (2)	2.1-5.0		
Nicotinic acid (μg/ml)	0.19	7 (6)	0.07-0.23	9.6	9 (6)	7.6-14.5		
Pantothenic acid† (µg/ml)	0.10	6 (4)	0.06-0.14	1.4	4 (4)	1.1-1.6		
Riboflavin (μg/ml)	0.58	2 (2)	0.42-0.41	9.2	8 (6)	5.8-10.2		

The numbers in parentheses refer to the number of bitches used.

no information could be found in the literature, blood and milk samples were taken from a number of fasting bitches. All milk samples were defatted before analysis.

Ascorbic-acid levels were determined by the method of Roe & Kuether (1943); B vitamins were estimated by standard microbiological procedures (Association of Vitamin Chemists, Inc. 1951).

Although, in most cases, blood and milk samples were drawn simultaneously from each animal, considerable variations in the ratio milk:blood-serum for any one vitamin were found. The ratios, however, differed radically for each vitamin, and all exceeded the fourfold concentration of ascorbic acid. This phenomenon may be found to be related to the vitamin requirements of the newborn animal.

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The effect of different fats on the retention of nitrogen in the rat. By D. J. NAISMITH and RAHMAT U. QURESHI, Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7

It has long been recognized, and frequently reported, that dietary fat, as an energy source, can influence the utilization of protein. The quantitative aspects of this relationship, for the rat, have been described by Munro & Naismith (1953). Recently Markova (1957) claimed that the quality of a dietary fat may affect its protein-sparing

^{*}Activity for Lactobacillus leichmannii ATCC 7830.

[†]Free vitamin only determined.

properties. Four fats, fed in isocaloric amounts to rats, were found to produce a response in nitrogen balance which could be inversely related to the degree of saturation of the fat. This finding could not be substantiated in the present experiments, which form part of a study of the nutritive value of the three fats most commonly used in the Pakistan dietary. A Pakistani-type diet, based on information from the Yearbook of FAO (FAO, 1957) was prepared, containing 10% ghee, hydrogenated cottonseed oil or mustard-seed oil. This diet was fed to rats in amount sufficient to permit growth, and nitrogen balance was determined for a 5-day period.

Coefficients of digestibility of fats and nitrogen balances (5-day period) of rats fed diets containing different fats

No. of rats	Nature of dietary fat	Iodine value	Coefficient of digestibility (%)	N	balance	(g)
10	Ghee	28	98.6		+0.450	
10	Hydrogenated cottonseed oil	69	98.2		+0.450	
10	Mustard-seed oil	104	95.2		+0.430	

Although the three fats used differed considerably in their degree of saturation, no statistically significant differences in nitrogen retention were found. The lower coefficient of digestibility of mustard-seed oil did not apparently reduce available energy by an amount that could produce a measurable effect on nitrogen balance.

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