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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Sixtieth Meeting of The Nutrition Society was held at the National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW 7, on Saturday, 5 October 1963, at 10 am, when the following papers were read :

Haemoglobin levels among Dayaks in Sarawak. By G. R. WADSWORTH, Sir John Atkin's Laboratories, Queen Elizabeth College, Campden Hill, London, W 8

Because the population is widely dispersed and geographical conditions difficult very little is known about the incidence of anaemia in the rural areas of Sarawak. The results of a limited survey in two different areas are therefore presented in this communication.

Estimations of haemoglobin concentration were made on capillary blood using the MRC grey-wedge photometer as previously described (Wadsworth & Lee, 1959).

Haemoglobin levels of Dayaks living in two different areas of Sarawak

Area	No.	Mean Hb concentration (g/100 ml)	Range (g/100 ml)	
Bunuk	Infants	24	10.6	7.1-13.6
	Toddlers	52	11.8	5.8-14.5
	Children	14	12.4	11.4-13.8
	Adult women	44	12.6	10.4-14.7
Lemanak	Infants	13	11.2	9.5-12.6
	Toddlers	64	12.1	7.1-15.1
	Children	128	12.9	8.1-16.9
	Adolescents:			
	boys	18	14.7	12.3-15.8
	girls	18	13.3	11.2-16.0
	Adult men	80	14.8	4.9-18.9
Adult women	82	12.5	5.5-16.0	

The results are shown in the table. Haemoglobin levels were often much lower than those accepted as normal, and the results indicated that between 1 and 2% of the populations tested had serious degrees of anaemia, that is haemoglobin concentrations of less than 7.5 g/100 ml.

REFERENCE

Wadsworth, G. R. & Lee, T. S. (1959). *J. trop. Pediat.* **5**, 67.

The different lipid response in adult males to dietary starch and sucrose.

By I. MACDONALD and DIANA M. BRAITHWAITE, *Physiology and Dietetic Departments, Guy's Hospital and Medical School, London, SE 1*

Seven healthy adult males were given a diet which consisted of 500 g of sucrose or raw maize starch daily, together with *ad lib.* quantities of lean meat or fish, green

vegetables, raw fruit, and 100 ml skim milk. The experimental diet was given for 25 days, followed by 25 days on a normal free-choice diet, with a final 25 days on a diet which contained the alternative carbohydrate.

Weekly samples of serum were analysed for the concentration of total lipid, serum lipid fractions and fatty acids. The fatty acid proportions in the depot fat were also estimated weekly.

The high-sucrose diet was associated with an increase in the serum lipid level, mainly in the glyceride fraction. The high-starch diet resulted in a fall in the serum lipid level, due to reduced concentrations of sterol ester and phospholipid fractions. The fatty acid pattern of the total serum lipid changed while on the diet and that for starch was different from that for sucrose. The fatty acids in the adipose tissue were also affected by the high carbohydrate intake and again the pattern differed between starch and sucrose in the diet.

We are very grateful to Beecham Food and Drink Division Ltd for a research grant.

The plane of nutrition and compensatory growth in pigs. By D. W.

ROBINSON, *Veterinary Field Station, University of Liverpool*

Many farm animals exhibit a remarkable recuperative capacity in growth after periods of underfeeding. This abnormally high rate of growth has been described as 'compensatory growth' and may occur under natural conditions following a period of limited food intake, or it can be induced experimentally by regulating the plane of nutrition. Similar effects of compensatory growth have been recorded incidentally in experimental work with the pig, where the quality of the ration and particularly the protein and energy levels have been the factors under consideration. A number of factors may influence the degree of compensatory growth made and some of these have been investigated in relation to the pig. Forty animals were used, five castrate males and five females on each of four treatments. The treatments were designed to provide varying degrees of restriction by reducing the nutrient intake over different live-weight intervals. Control pigs were restricted to a scale approximating appetite and the other groups were allowed 75% of the intake of controls to 80, 120 and 160 lb live weight respectively after which they were fed the same as controls.

The relative growth rates for the various live-weight intervals have been calculated from the mean growth curves using Brody's (1945) instantaneous relative growth rate (R) equation, in the integrated form giving the following relationship:

$$R = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

The results indicated that on all treatments some degree of compensatory growth was made when the restrictions were lifted. Pigs only slightly restricted grew faster than controls later on in development in spite of the absence of an immediate response. The immediate response in growth rate appeared to be directly proportional to the duration of restriction and in all cases gilts responded more than

male castrates following realimentation. In spite of the marked degree of compensatory growth in some treatments, in no case did the animals fully compensate for periods of restriction and the overall results indicated that controls grew the quickest. The results also showed that even growth rates, such as those of the control animals and interrupted growth, were equally efficient in relation to food intake per unit of gain.

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The effects of hookworm infestation (*Nippostrongylus muris*) on protein metabolism in rats. By R. ORRACA-TETTEH and B. S. PLATT, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Three experiments were made, each with six groups of four rats 30 days old. Low- and high-protein diets (NDpCal% = 4.9 and 13.3) were given and the test animals were infected with 250 larvae (see Orraca-Tetteh & Platt, 1964). The plan of the experiments is shown in the table.

Effect of Nippostrongylus muris infestation of rats on NPU and NDpCal%

Treatment	Low-protein diet		High-protein diet	
	NPU _{OP}	NDpCal%	NPU _{OP}	NDpCal%
Infested; fed <i>ad lib.</i>	35.3	2.1	41.6	11.8
Uninfested; pair-fed	71.2	4.3	44.3	12.5
Uninfested; fed <i>ad lib.</i>	81.1	4.9	47.2	13.3

The net protein utilization (NPU) (Miller & Bender, 1955) of the infested animals was lower than that of the uninfested animals. The infested animals on the low-protein diet showed greater reduction in NPU than those on the high-protein diet. Among the uninfested animals on the low-protein diet, those pair-fed had a somewhat lower NPU than those fed *ad lib.*, indicating that reduction of appetite on infestation did not contribute greatly to the reduction in NPU.

Infested animals showed a lowered apparent digestibility of nitrogen, which was greater in those on the low-protein diet than in those on the high-protein diet. The lowered apparent digestibility of nitrogen does not account for all the reduction in protein utilization in infested animals.

Total body water was greater and body fat content lower in infested animals on both diets than in the controls. The body protein content of infested animals on the low-protein diet was less than that of controls, but there was no difference in the animals on the high-protein diet.

The livers of infested animals on both diets were heavier than those of uninfested controls and had higher water, fat and nitrogen contents, but the spleens were lighter and more congested. The haemoglobin concentration and packed cell volume in the infested animals rose during the course of infestation, but later fell below the levels for uninfested animals.

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Effect of chronic roundworm infection (*Toxocara canis*) on protein metabolism in dogs fed diets of different protein value. By H. A. AL-RABII and B. S. PLATT, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Platt, Heard, Stewart & Al-Rabii (1962) reported some biochemical changes resulting from feeding diets of inadequate protein value to dogs. The animals all acquired (pre- or neo-natal) infection with *Toxocara canis*; they therefore provided an opportunity for studying the effects of the protein value of the diet and infection.

Diets of different protein value (see table) were used. All the dogs consumed the same quantity of food; therefore any effect of infection on the protein value of the diet was not due to caloric restriction (Platt, Miller & Payne, 1961). The animals were treated with piperazine hexahydrate (100 mg/kg body-weight) thrice weekly until worm-free. Reinfection was by oral administration of 500 embryonated eggs. Egg counts were made at weekly intervals.

Nitrogen balance and apparent digestibility were measured for 3 successive days at regular intervals. The results given in the table are for thirty-six animals of 4-6 months of age when infection was at its peak.

NdpCal% of diet*	Nitrogen-balance (g/24 h)		Nitrogen digestibility (%)		Eggs (no./g stool)
	Dewormed	Infected	Dewormed	Infected	
14.0 (4)	1.14	0.88	88	82	4 × 10 ²
8.6 (8)	0.98	0.55	82	75	7 × 10 ²
6.8 (8)	0.60	0.14	71	68	5 × 10 ²
12.0 (8)	1.08	0.83	86	83	4 × 10 ²
6.3 (8)	0.65	0.18	70	66	4 × 10 ³

*No. of animals given in parentheses.

Nitrogen balance was reduced only slightly in infected dogs fed diets of high protein value, but was severely reduced in litter-mates on diets of lower protein value. This impaired nitrogen balance was not primarily due to changes in digestibility, which was only slightly reduced by infection; it was due rather to increased protein katabolism, as shown by increased excretion of urinary nitrogen. There is evidence from the reduced rate of growth, anaemia, increased water and fat in liver and muscle, and changes in serum protein fractions (Al-Rabii, 1964) that the effect of this loss of nitrogen consequent on infection is to intensify the dietary protein-calorie deficiency, especially in the animals on diets of low protein value. Conversely, dogs fed on diets of high protein value were more resistant to infection and more easily dewormed than dogs on diets of inadequate protein value.

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The effect of environmental temperature on net protein utilization and food intakes of rats. By M. JACOB and P. R. PAYNE, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

When animals are exposed to environmental temperatures below the range of thermal neutrality, resting heat production rises and this is accompanied by an increase in voluntary food intake. Thus a reduction in environmental temperature may cause an animal to increase its intake of protein, and it is important to know whether this extra protein is available for anabolism, or is simply used as a source of energy.

There are conflicting reports in the literature about the effect of cold exposure on growth rate in young animals: thus Sellers (1957) and Beaton (1963) found increased food intakes, but no improvement in growth performance, with reduction in environmental temperatures, whereas Treadwell, Flick & Vahouny (1957) observed higher growth rates and protein efficiency ratios at 1° than at 25°, when the protein content of the diet was 5 or 10%, but the reverse effect with a diet containing 25% protein.

In this study, the food intakes, net protein utilization (NPU) and net dietary calories % (NDpCal %) of diets containing 0, 4, 10 and 25% casein were measured, using rats housed at temperatures of 25° and 15° for a 10-day period, and the values obtained are shown in the table.

Diet	NPU	NDpCal %	Caloric intake (kcal/day kg ^{0.75})	Weight gain (g/rat day)
4% casein 25°	76	3.2	171	-0.1
4% casein 15°	64	2.7	237	+0.2
10% casein 25°	71	7.4	237	1.8
10% casein 15°	71	7.4	332	2.6
25% casein 25°	54	13.6	250	4.1
25% casein 15°	36	9.0	322	3.2

At all levels of protein, food intakes were higher at 15° than at 25°; however the rate of loss of nitrogen from the rats fed the non-protein diet, and the NPU of the 4% and 10% diets were the same at both temperatures, i.e. for these animals, the extra protein consumed was utilized for anabolism at the same efficiency as at normal temperatures, and this was reflected in an improved growth performance. However, the NPU and NDpCal % of the diet containing 25% casein were considerably lower when fed at 15° than at 25° and a reduced growth rate was observed. This reduction in protein value, and also the further reduction which was observed when the same diet was fed in restricted amounts, could be explained by the inadequacy of the caloric intakes of the animals in relation to their energy needs for basal metabolism, increased heat production and growth. The observed protein values are satisfactorily predicted by the equation

$$\text{NDpCal \%} = 17 \left(1 - \frac{70 + H}{C} \right)$$

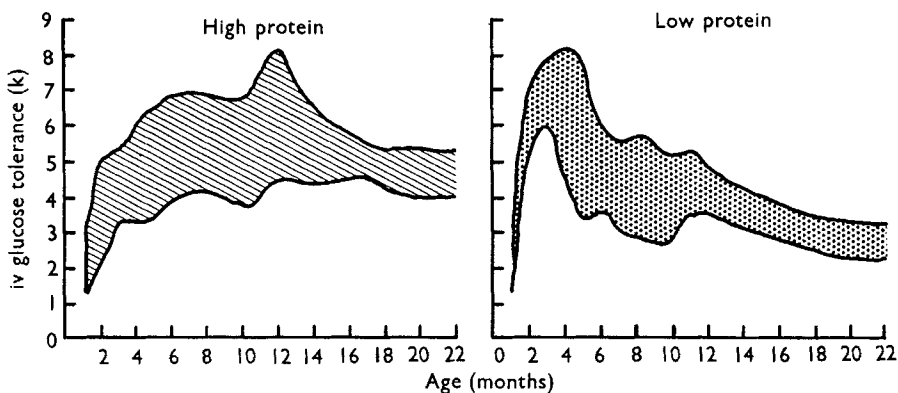
where H=heat required for maintenance of body temperature, and C=caloric intake in kcal/day (kg body-weight)^{0.75}.

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Diabetic-like changes in carbohydrate metabolism in dogs fed diets of suboptimal protein value. By C. R. C. HEARD, M. R. TURNER and B. S. PLATT, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Glucose tolerance (K) changes with age both in dogs fed diets the protein value of which was adequate (NDP Cal=9-10%) for all stages of growth and in litter-mates receiving diets inadequate (NDP Cal=6.5%) for optimal growth, pregnancy and lactation (see figure). The increase in K in early life occurs also in pigs (Heard, Durbin & Platt, 1961). Protein-calorie deficiency accelerates and exaggerates this increase in K, which is followed, at 6 months of age, by impaired glucose tolerance (Platt, Heard, Stewart & Al-Rabii, 1962). At this stage insulin (0.1 i.u./kg), either alone or with glucose (0.4 g/kg i.v.), produces in deficient animals a slower and less marked fall in blood glucose concentration than that found in adequately fed litter-mates. Restoration of blood glucose concentrations after hypoglycaemia occurs at the same rate in both groups of dogs.



Changes in glucose tolerance (K) with age in dogs fed diets of different protein value. (K, as defined by Ikkos & Luft, 1957).

In 7-month-old protein-calorie deficient dogs, plasma insulin-like activity (ILA), following intravenous glucose, is low as measured by the rat diaphragm technique but normal to elevated as measured by the rat adipose tissue technique. This association of an early phase of hyperinsulinism followed by insulin resistance, elevated 'total' ILA activity and impaired glucose tolerance is also encountered in adult-onset diabetes, prediabetes and obesity (Gordon, 1962). The similarity between marginal protein-calorie deficiency and diabetes extends to the offspring

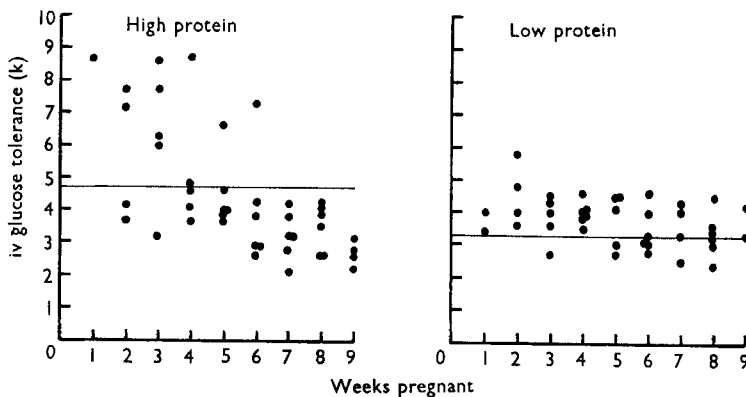
(Turner, Heard & Platt, 1964). The fact that 'impaired' glucose tolerance in dogs ($K=3.3$ at 20 months) is still higher than that found in 'normal' humans ($K=1.6$; Lundbaek, 1962) may account for the absence, in deficient dogs, of significantly elevated fasting blood glucose or non-esterified fatty acid levels.

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 Turner, M. R., Heard, C. R. C. & Platt, B. S. (1964). *Proc. Nutr. Soc.* **23**, vii.

The effects of mild protein-calorie deficiency on the carbohydrate metabolism of pregnant bitches and their offspring. By M. R. TURNER, C. R. C. HEARD and B. S. PLATT, *Human Nutrition Research Unit, National Institute for Medical Research, London, NW 7*

Bitches which had been weaned on to mildly protein-calorie deficient diets (NDpCal=6.5%) were mated at approximately 20 months of age. The effects of pregnancy on glucose tolerance of the deficient and control animals (NDpCal=9-10%) are shown in the figure. Changes in glucose tolerance similar to those of the control and deficient bitches have been observed in normal and prediabetic women respectively (Bastennie, Conard, Franckson, Bellens & Malaisse, 1963).



Glucose tolerance (K) during pregnancy in dogs receiving diets of different protein value.

Prior to mating, a standard intravenous dose of insulin (0.1 i.u./kg) caused in deficient bitches a slow small fall in blood glucose and a slightly reduced rate of recovery from hypoglycaemia. In late pregnancy both control and deficient bitches had similar insulin tolerance curves characterized by a slow fall in blood glucose to a lower level than in non-pregnant animals, and a slow recovery from hypoglycaemia.

The exaggerated and accelerated increase in glucose tolerance observed in normally born pups 2 months after weaning on to a deficient diet, was already apparent at weaning age (6 weeks) in the offspring of deficient bitches ($K=7.7$). At 6 weeks they

also had elevated liver glycogen levels and an increased sodium : potassium ratio. The children of diabetic mothers have at birth a high tolerance to glucose and hyperinsulinism (Baird & Farquhar, 1962).

Although pregnancy is one of the factors thought to accelerate the onset of overt diabetes mellitus, the effect of several pregnancies on the deficient bitches was to alleviate rather than worsen the 'diabetic-like' state, possibly as a result of a compensatory alteration in anterior pituitary function.

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The role of the essential fatty acids in the aetiology of kwashiorkor. By D. N. NAISMITH, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

The minimum daily requirement of the essential fatty acids (EFA) by the human infant has been estimated as 1% of the total caloric intake (Wiese, Hansen & Adam, 1958). A deficiency of EFA might arise naturally through inadequate nutrition of the mother, resulting in the production of milk low in linoleic acid, or through the feeding of diets low in fat.

In Nigeria, where kwashiorkor is endemic, the infant, during the pre- and post-weaning periods is fed on starchy paps prepared from foodstuffs containing negligible amounts of fat. Samples of these preparations, and of breast milk from Yoruba women, have been analysed, and children with severe kwashiorkor examined for biochemical evidence of EFA deficiency. In the foodstuffs *eko* (extracted maize), *eba* (cassava) and *amala* (yam) fat represented less than 0.5% of the total solids. Breast milk, in contrast, was rich in EFA; linoleic acid accounted for 15.1% of the fatty acids (7.6% of the total calories) of milk.

Blood was taken from twenty patients, aged between 2 and 3 years, and the composition of the serum lipids determined using an argon gas chromatograph. Blood was also obtained from twenty normal healthy Yoruba children, between 9 and 18 months of age, for comparison of the pattern of fatty acids. The amounts of linoleic acid, arachidonic acid and eicosatrienoic acid in the serum of patients with kwashiorkor, expressed as a percentage of the total fatty acids, were 20.5, 3.6 and 3.9 respectively. In the control group, the values were 27.4, 8.6 and 2.0.

It has been suggested that a ratio of eicosatrienoic acid to arachidonic acid in excess of 0.4 is indicative of EFA deficiency (Holman, 1960). The ratio of these fatty acids in the serum of the group with kwashiorkor was 1.08, and in the control group 0.22.

A dietary deficiency of EFA can lead to a severe impairment in the utilization of protein (Naismith, 1962). It is therefore suggested that, owing to a lack of linoleic acid during the postweaning period, the child with kwashiorkor may waste a considerable part of the limited amount of protein in its diet.

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Interactions between copper, zinc and iron and their relationship to copper toxicity in the pig. By N. F. SUTTLE and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen*

Although the beneficial effects of supplementing pig rations with 250 p.p.m. copper have been conclusively demonstrated (Braude, Townsend, Harrington & Rowell, 1962), infrequent reports of the toxicity of such supplements have limited the wider use of Cu. Dietary mineral imbalance could conceivably contribute to the variable responses to Cu supplements. The effects of adding zinc and iron supplements of a maize-soya-bean meal diet containing a toxic concentration of Cu were studied in two separate experiments of 6 weeks' duration with the following results:

Supplement	Haemoglobin after 6 weeks (g/100 ml) Mean (6)	Serum Cu after 4 weeks (μ g/100 ml) Mean (6)	Serum aspartate transaminase activity after 4 weeks ($10^3 \Delta E_{340}$ of NADH ₂ /min ml) Mean (6)
		Expt 1	
o	10.4	117	1.52*
500 p.p.m. Zn (as ZnCO ₃)	9.6	—	—
750 p.p.m. Cu (as CuCO ₃ , Cu(OH) ₂)	7.8	388	2.12*
500 p.p.m. Zn + 750 p.p.m. Cu	7.6	225	1.71*
SD	± 0.56	± 88	$\pm 0.26^*$
Overall significance	0.1%	1%	1%
		Expt 2	
o	9.6	155	29.8
750 p.p.m. Fe (as FeSO ₄ · 7H ₂ O)	10.3	192	34.5
750 p.p.m. Cu (as CuCO ₃ , Cu(OH) ₂)	6.7	279†	62.2†
750 p.p.m. Fe + 750 p.p.m. Cu	8.4	184	30.0
SD	± 1.0	± 41	± 4.1
Overall significance	1%	NS	NS

N.S., not significant.

*Logarithmic values.

†Values excluded from statistical analysis.

The addition of Cu in the absence of Zn or Fe caused serum Cu and transaminase levels to rise rapidly to a maximum after 4 weeks. Generally there was jaundice at this time, but this disappeared as the serum Cu and transaminase values declined in the following weeks despite maintenance of supplementation. Two pigs in Expt 2 showed no toxic responses to 750 p.p.m. Cu.

The addition of Zn or Fe to the high Cu ration lessened the increase in serum Cu and transaminase values and eliminated jaundice. Only the Fe supplement gave protection against the anaemia associated with the high Cu intake. Both Zn and Fe

supplements therefore gave some protection against the toxicity of 750 p.p.m. Cu, and it is possible that much lower concentrations of Cu may also be toxic where dietary Zn and Fe are low.

One of us (N.F.S.) would like to acknowledge the receipt of a Pig Industry Development Authority research scholarship which made this work possible.

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Vitamin A and riboflavine concentrations in livers of intensively fattened cattle. By J. QUARTERMAN and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen*

Recent work on the use of all-concentrate rations for fattening cattle (Preston, Aitken, Whitelaw, McDearmid, Philip & MacLeod, 1963) is, in many instances, being successfully applied in commercial practice. However, a high proportion of animals fattened on this type of ration exhibit liver and kidney damage at slaughter and occasional cases of blindness have been reported. The possible nutritional origin of these lesions prompted a study of the vitamin content of liver tissue from cattle given high-energy, low-fibre rations.

Evidence that feeding 'high-energy' rations decreases liver stores of vitamin A (Erwin, Gordon & Algeo, 1963), and that a diet containing 30% of soya-bean meal had the same effect in a study carried out by Shaw, Moore & Sykes (1951) led us to examine liver vitamin A concentrations. Since the synthesis of B vitamins in the rumen is influenced by the nature of the diet the riboflavine content of liver was also examined.

Cores of liver were taken at slaughter from animals in fat condition fed on 'all-concentrate' rations and from animals fed on winter fattening rations traditional in NE Scotland (silage, hay or oat straw, turnips and oats or protein concentrates). There was no significant difference in riboflavine content of the livers between the two treatments. The mean concentration of vitamin A in livers from animals fattened on traditional rations was nearly twenty times greater than that of animals fed on the 'all-concentrate' ration despite the routine inclusion of vitamin A in the latter ration to provide approximately 24 $\mu\text{g}/\text{kg}$ live weight daily. It has been previously suggested that 5 $\mu\text{g}/\text{kg}$ live weight daily is adequate to meet requirements ((USA) National Research Council, 1958). Differences in β -carotene and xanthophyll concentration were smaller but showed the same trend. Of the fifteen livers examined from animals of the 'all-concentrate' group, seven had liver vitamin A concentrations below the lower limit of the normal range of 0.9–4.2 mg/100 g suggested by Moore & Payne (1942) and five had concentrations within the range of 0–0.077 mg/100 g previously associated with clinical signs of vitamin A deficiency in cattle (Dehority, Hazzard, Eaton, Grifo, Rousseau, Helmboldt, Jungherr & Gosslee, 1960). The implications of this finding are being examined.

Table 1. *The mean vitamin A, carotene, xanthophyll and riboflavine content of fat cattle at slaughter*

Nature of ration Age at slaughter	'All-concentrate' 11-13 months		'Traditional' 24-30 months	
	mg/100 g	No. of samples	mg/100 g	No. of samples
Vitamin A	0.43 ± 0.15*	15	7.69 ± 1.63*	10
β-carotene	0.19	3	0.54	2
Xanthophylls	0.03	3	0.10	2
Riboflavine	2.8	3	3.3	2

*Standard error of the mean.

We are indebted to F. Hoffman-La Roche & Co., Basle, for undertaking analytical work in this experiment.

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The effect of intramuscular iron injection on growth rates and haemoglobin levels of outdoor-reared piglets. By G. A. LODGE and B. HATTON, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough*

The intramuscular injection of iron-dextran or iron-dextrin complexes is now widely practised in the prevention of piglet anaemia. Walker & Taylor (1961) obtained a growth response in piglets given 200 mg Fe as ferric oxide dextrin over those given 150 mg Fe as ferric hydroxide dextran, although there was no difference in mean haemoglobin level between the two treatment groups. On the other hand, Crawley & McDiarmid (1959) gave injectible iron to outdoor-reared piglets and obtained significantly higher haemoglobin levels but no growth response.

In the present experiment, one hundred piglets in twelve litters were reared on their dams to 59 days of age in fold units on grass. At 3 days of age one half of each litter was left untreated while the other half was given 200 mg Fe/piglet in the form of 2 ml iron-dextran (Imposil 200; Bengel Laboratories Ltd, Holmes Chapel, Cheshire) injected into the muscles of the thigh. Throughout the experiment all pigs were weighed weekly and blood samples taken from an ear vein at 3, 10, 24, 52 and 59 days of age. Supplementary feed was given *ad lib.* from 3 weeks of age.

Differences in weight between the two groups were greatest from 31 days until weaning, during which time there were no significant differences between groups in mean haemoglobin values. This suggests either a carry-over effect from earlier deficiency, or the existence of a deficiency of iron for maximum growth in pigs with

access to iron from natural sources which exerts its effect through tissues other than blood.

Growth rates and haemoglobin levels of outdoor-reared piglets with and without iron-dextran injection at 3 days of age

Age (days)	Mean weight		Significance of difference <i>P</i>	Mean haemoglobin level		Significance of difference <i>P</i>
	With Fe (lb)	Without Fe (lb)		With Fe (g/100 ml)	Without Fe (g/100 ml)	
3	3.8	3.6	NS	10.5	10.4	NS
10	7.4	6.9	0.01	10.5	9.5	0.001
17	11.1	10.5	NS	—	—	—
24	14.8	13.9	NS	12.5	11.6	0.001
31	19.7	18.3	0.01	—	—	—
38	25.6	24.0	0.05	12.1	12.0	NS
45	32.2	30.5	0.001	—	—	—
52	40.3	37.3	0.001	12.1	11.9	NS
59	47.9	45.4	0.05	12.2	12.0	NS

NS, not significant.

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The influence of dietary protein level on deposition of cholesterol in the tissues of the cholesterol-fed rabbit. By H. N. MUNRO, W. FORBES and MARIAN H. STEELE, *Departments of Biochemistry and Physiology, University of Glasgow*

When rabbits are fed for several weeks on a stock diet with 1% cholesterol added, they develop enlargement of the adrenal glands, due to an increase in the size of the cells of the cortex in which there is extensive deposition of cholesterol (Forbes, Steele & Munro, 1964). Adrenal gland size is also influenced by the protein content of the diet (Munro, Hutchison, Ramaiah & Neilson, 1962). It was therefore of interest to determine whether the action of administered cholesterol on the adrenal gland could be modified by changing the protein intake of the rabbits.

Rabbits of about 2 kg body-weight were fed for 13 weeks on a fixed adequate intake of diets containing either 8% or 33% protein made by exchange of casein for dietary starch. Addition of 1% cholesterol to the diet caused a similar degree of adrenal enlargement at each level of protein intake, but deposition of cholesterol in the adrenal cortex was much greater when the diet contained 33% protein.

Examination of the livers of these animals showed a similar effect of dietary protein level on deposition of cholesterol in the hepatic cells. This difference in

cholesterol deposition was found in animals fed these diets for intervals as short as 2 weeks. The amounts of protein, phospholipid and ribonucleic acid in the liver cell were not affected at either level of protein intake by addition of cholesterol to the diet.

Examination of the aortas of rabbits fed on these diets for long periods showed that an elevated intake of protein tended to reduce the frequency of spontaneous atherosclerosis. When cholesterol was added to the diet, however, the incidence of cholesterol atherosclerosis was accentuated by a high intake of protein. This has also been observed by Lofland, Clarkson & Goodman (1961) in pigeons fed on protein-rich diets containing added cholesterol.

These findings indicate that the effect of dietary cholesterol on various tissues of the body can be modified by level of protein intake.

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The efficiency of utilization of limiting amino acids: some sulphur and nitrogen balances. By G. DONOSO, *School of Medicine, Chile*, D. S. MILLER, *Queen Elizabeth College, Campden Hill Road, London, W8*, and P. R. PAYNE, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

It has been shown that the efficiency of nitrogen utilization declines as the concentration of protein in the diet is increased. This reduced efficiency is not due to a saturation of the animal's anabolic capacity since the growth rate of animals progressively increases with protein level. Thus, growth is essentially a wasteful process at least for protein. Results of experiments are presented to show that even the most limiting amino-acid of a diet is subject to the same lowered efficiency of utilization. Diets containing 10, 15, 25, and 35% casein were fed to weanling rats and the net utilization of both N and sulphur were determined according to the procedure of Miller & Bender (1955) and both these values declined as the concentration of casein in the diets increased. The utilization of sulphur was taken to indicate the fate of methionine and cysteine which are the limiting amino-acids in casein, and high values were obtained at the low protein levels, falling to an efficiency of only 50% when the diet contained 35% casein. However even at the highest protein level, an improved growth rate was obtained by adding more methionine to the diet.

It follows that it is impossible to state the amino acid requirements for a given performance in terms of g/day. To demonstrate this, two diets both limited by the sulphur amino acids were constructed; one contained 16% casein (score=70) and the other 28% protein from groundnut (score=50). When fed to rats in equal amounts these diets supported identical weight and N gains despite quite different daily intakes of the sulphur amino acids (35 and 40 mg/day) and sulphur (7.0 and

11.2 mg/day). It would seem that the pattern of amino acids in relation to the rest of the diet is more important than the amounts consumed per day.

The results of these experiments may be explained on the hypothesis that proteins, essential amino acids, and even limiting amino acids are katabolized in proportion to their concentration in the diet, and this is consistent with our theory of protein metabolism (Miller & Payne, 1963).

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Energy expenditure and body build in sixty 14-year-old children. By J. V. G. A. DURNIN, W. J. McLEES, S. A. YUILL, A. D. BUSBY, C. A. GAY and E. C. BLAKE, *Institute of Physiology, University of Glasgow*

Measurements have been made of energy expenditure and 'body build' on thirty boys and thirty girls, aged 14 years, living in Glasgow. Energy was determined during 7 days by the 'diary' technique and indirect calorimetry (Durnin & Brockway, 1959). Body build was assessed from standing height, subcutaneous thicknesses at the biceps, triceps, subscapular and supra-iliac regions, and gross body-weight. The mean heights of the boys and girls were similar at about 160 cm, mean weights were both about 50 kg, but the boys expended 2800 kcal/day of energy whereas the relevant value for the girls was 2200 kcal/day. No high correlation coefficients were found for either weight and energy expenditure, height and energy or the sum of the four subcutaneous thicknesses and energy. As might be expected at this age, the boys were not so uniformly mature, in the physical developmental sense, as the girls and the five boys who were considerably less developed than the remainder were thinner, lighter in weight and expended about 600 kcal/day less than the other boys. No such differences appeared among the girls.

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Further observations on the interaction of protein and calcium on the bones of rats. By N. R. H. EL-MARAGHI and R. J. C. STEWART, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Ninety rats were given a stock diet until they were 28 weeks of age. They were then transferred to individual cages and maintained for 20 weeks on diets having protein values of $\text{NDP Cal}\% = 6.0$ or 1.6 . Diets containing 0.44 and 0.11% of calcium were

prepared at each protein value, and eight rats (four ♂ and four ♀) were maintained on each of the four diets. Two further groups on the diets of higher protein value were pair-fed (PF) the amounts eaten by the protein-deficient animals. The remainder of the rats were given the low-protein, low-Ca diet for 20 weeks and then extra Ca, protein or both Ca and protein for a further 10 weeks. All the animals received vitamin mixtures and were given distilled water to drink.

There was some increase (13%) in the body-weight of the rats given the diets of higher protein value *ad lib*. Those receiving the diets of low protein value lost about 38% of their original weight and the pair-fed animals about 18%.

The bones of the animals receiving the diets of higher protein value were radiographically denser than those of the protein-deficient animals. On the other hand, only minor changes were brought about by the different Ca intakes. The ash per cm³ of the femur is shown in the table; the values are consistent with the radiographic appearance.

Ash content (mg/cm³) of bone (femur) of rats

	Male				Female			
	After 20 weeks on diet		After 10 weeks 'recovery'		After 20 weeks on diet		After 10 weeks 'recovery'	
Ca in diet (%)	0.44	0.11	0.44	0.11	0.44	0.11	0.44	0.11
NDpCa%: 6.0	633	615	567	536	679	651	580	551
1.6	534	472	426		527	484	459	
6.0 (PF)	586	549			621	569		

Of the animals whose diet was changed after 20 weeks on the low-protein, low-Ca regimen those which continued on the low-protein ration with an increased supply of Ca had lower ash concentrations than those killed at 20 weeks. Improving the protein value of the ration brought about a return to the original (28 week) body-weight and an increase in the ash concentration in the femur, the latter being greater when both protein and Ca were supplied.

These results for adult animals, in which increases in the length of the bones were insignificant, emphasize the importance of an ample supply of protein as well as Ca for the maintenance of bone tissue.

The quantitative estimation of lipids in nervous tissue, by thin-layer chromatography. By SHEILA N. PAYNE, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Lipids from freeze-dried nervous tissue are extracted with CHCl₃-MeOH (2 : 1, v/v), and an aliquot of this extract, equivalent to 100-200 μg lipid, is applied to a thin layer of Kieselgel G supported on a glass plate. The plates are run in the solvent system of Jatzkewitz (1961), then sprayed with 50% H₂SO₄ containing 5 mg% methyl orange and heated for 20 min at 160°.

The extinction of the separated lipids on the plate is measured at intervals of 0.3 mm by a reflectance scanner. The amount of each lipid fraction present is proportional to the area enclosed by the various curves obtained.

The table shows the reproducibility of the method. With pure lipids as standards it is possible to estimate absolute amounts present in tissues.

Amount of lipid applied (μg)	194	151	109*	85	60
Lipid	Area under curve as % of total area				
Ganglioside a+b	0.7	1.0	0.6 \pm 0.05	0.3	0.5
c	1.3	2.0	1.3 \pm 0.07	0.8	1.0
d	0.8	1.3	0.7 \pm 0.09	0.4	0.5
Lysocephalins and lysolecithin	0.8	1.3	1.4 \pm 0.02	1.4	1.6
Phosphatidyl serine	6.2	9.0	8.4 \pm 0.78	9.3	10.3
Sphingomyelin	9.0	6.3	7.3 \pm 0.34	4.1	3.8
Lecithin	19.4	18.9	18.9 \pm 0.41	18.3	18.9
Cerebroside sulphuric acid ester	7.5	6.6	6.0 \pm 0.41	4.8	4.8
Phosphatidyl ethanolamine	32.4	31.4	33.2 \pm 0.59	35.3	35.2
Degradation product of phosphatidyl ethanolamine	2.4	2.8	2.7 \pm 0.45	2.5	4.3
Cerebrosides (phrenosin and kersasin)	20.4	19.9	20.4 \pm 0.45	22.6	19.0
Total area under curves (in^2)	38.59	36.70	29.74 \pm 0.7	22.71	16.73
Wet weight of brain tissue (mg)	2.09	1.63	1.18	0.92	0.65

*All values given in this column are the means and standard errors of five separate determinations on three different plates on 3 different days.

This method is simpler and more convenient than those of Jatzkewitz (1961) and Davison & Graham-Wolfaard (1963), in which the lipids are scraped off the plate before estimation.

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Cytological changes in the gastro-intestinal tract of protein-calorie deficient animals. By B. T. SQUIRES, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Shorr's (1941) staining technique for vaginal smears can also be applied to sections of the epithelium of the gastro-intestinal tract, when the colours produced in vaginal smears, green for non-cornified cells and brown for cornified cells, still obtain. It has been constantly observed that the dichromatic picture varies in colour proportion roughly according to the protein-calorie content of the diet.

In sections of healthy young canine and murine stomach and gut, the green-stained area is much more extensive than the brown and the reverse obtains in sections from animals suffering from moderate to severe protein-calorie deficiency. If the preliminary stage of staining with haematoxylin is omitted, green and brown nuclear staining can be followed more easily. The green stain tends to occur uniformly throughout the section whereas the brown stain tends to concentrate noticeably along the villi and the lamina propria. In extreme cases of protein-calorie deficiency, there is little or no green colour left. This colour change can be roughly correlated with similar ones in the buccal mucosa, a scraping from which in health shows the proportion of young green* non-cornified cells with green nuclei at 60-70%, in protein-calorie deficiency this proportion drops to *c.* 25% or less, the cells with green cytoplasm and nuclei being largely replaced by cells with green cytoplasm but red-stained nuclei and particoloured transitional cells, and in extreme

*The term 'green' indicates 'green-stained'.

protein deficiency, no green cells will be seen. The buccal mucosa can thus be used to monitor changes in those parts of the gastro-intestinal tract in the intact animal which are susceptible to examination only by jejunal biopsy or enterectomy.

Other stains such as the FALG group (Gurr & MacConaill, 1960) or Gurr's keratin stain (Gurr, 1958) will also give analogous colour differentiation.

In children, healthy, moderately malnourished, and marasmic, comparable results have been obtained from buccal mucosa and the method has been checked against clinical findings in South Arabia and Basutoland, with consistent results.

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Starch-gel electrophoresis of serum protein of dogs: the effects of diets of differing protein value and of infection with *Toxocara canis*. By H. A. AL-RABII, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Paper electrophoresis has been used by Kagan & Goodchild (1961) for studying the mechanism of the pathogenesis of infections and the host response to them.

The object of the present study was to determine changes occurring in the relative proportions of serum protein fractions in dogs by starch-gel electrophoresis (Smithies, 1955) at regular intervals following single infection with 500 embryonated larvae orally administered. Litter-mates were fed on four diets of different protein value. The table shows concentrations of several serum protein fractions in dogs of 4-6 months of age. The effects of infection are most marked at this time which coincides with extensive oviposition.

Blood serum protein fractions (g %) of dogs, dewormed and infected, fed on diets of different protein value

NDP Cal % of diet No. of dogs	14.0		6.8		12.0		6.3	
	4		8		8		8	
	D	I	D	I	D	I	D	I
Total protein	7.25	6.55	5.35	4.80	6.85	5.10	5.00	3.90
Albumin	4.60	3.70	2.80	1.85	4.35	2.25	2.70	1.60
α -1-globulin	0.25	0.25	0.15	0.10	0.25	0.10	0.25	0.10
α -2-globulin	0.50	0.60	0.70	0.70	0.50	0.70	0.45	0.60
β -globulin	1.00	0.75	0.40	0.20	0.95	0.50	0.35	0.15
γ -globulin	0.90	1.25	1.30	1.95	0.80	1.55	1.25	1.45

D, dewormed; I, infected.

The usual effects of diets of low protein value on serum protein fractions were observed, namely reduction in total serum protein, involving principally albumin and β -globulins, but an increase in γ -globulins.

Similar changes were found in infected animals and these were more marked in dogs in which dietary protein-calorie deficiency and infection were combined.

Changes in the α -1- and α -2-globulin fractions were small, but there appeared to be a tendency for α -2-globulin to increase during infection.

Since γ -globulin concentrations were increased by feeding diets of low protein value to dewormed animals, such an increase in the same fractions observed in the infected dogs could have been due to an intensification of the general effects of protein-calorie deficiency as well as to increased antibody production (Al-Rabii & Platt, 1964).

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The use of *Nippostrongylus muris* and its effect on the intestine of the rat. By R. ORRACA-TETTEH and B. S. PLATT, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW 7*

Larvae of *Nippostrongylus muris* from 13–15-day-old cultures are suspended in distilled water and a volume containing the required number is placed directly on the exposed but unshaven skin of the rat. The rat is held for about 5 min during which the larvae penetrate through the skin; they then go via the lymphatic system to the blood stream, into the lungs, which they penetrate to reach the alimentary canal via the oesophagus. In the jejunum and upper ileum, they develop into the adult form. Egg production starts 6 days after penetration of the skin. The worms bury their heads in the epithelial layer of the villi and the epithelium is eroded where they lodge; the intestine enlarges and fills with gas and fluid. The animals on the low-protein diet showed greater enlargement of the intestine than those on the high-protein diet. The water content of the intestine (wall and contents) from the duodenum to the end of the ileum was higher in the infested animals than in the uninfested; also the infested animals on the low-protein diet had a higher water content than the infested animals on the high-protein diet.

In infested animals the muscular coats of the intestine hypertrophy, the villi are swollen and engorged with blood and the lamina propria is infiltrated with blood cells. At the height of the infestation, the villi shorten and the crypts become deeper. Earlier in the infestation, the goblet cells increase in number and are filled with mucin, but later they become empty, having discharged their contents. Heavy infestation always causes almost complete loss of the villi in the parts of the intestine affected.

Segments of excised duodenum from infested animals showed irregular movements, with alternating periods of contractions with high amplitude and quiescence. These differed from the regular rhythmic contractions of the excised duodenum from uninfested animals.

The total serum proteins were lower in the infested animals on both the low- and high-protein diets. There was a reduction in the serum albumin, hence a lowering of the A/G ratio in the infested animals.

Starch-gel electrophoresis of the serums showed a reduction in the β -globulin (transferrin) fractions and gross reduction in the fast-moving α_1 and α_2 fractions as the infestation progressed.

Effect of dietary protein on hepatic fibrogenesis in albino rats. By U. N. BHUYAN, N. C. NAYAK, M. G. DEO and V. RAMALINGASWAMI, *Department of Pathology, All India Institute of Medical Sciences, New Delhi, India*

The role of protein malnutrition in cirrhosis of the liver in man is largely controversial (Davies, 1950; Waterlow & Bras, 1957; Higginson, Grobbelaar & Walker, 1957). In rats cirrhosis of the liver can be quickly produced and the effect of dietary protein on hepatic fibrogenesis conveniently studied. Sriramachari (1958) observed that the development of carbon tetrachloride-induced cirrhosis is markedly inhibited in rats fed a low-protein, low-choline diet. He attributed this beneficial effect to low protein.

Young albino rats of both sexes, weighing 100 g on average were divided into two groups and force-fed by gastric tube twice a day. Group 1 received a high-protein diet containing 16% casein and group 2 a low-protein diet with 3% casein. Carbon tetrachloride was administered by the oral route biweekly to all animals in calculated doses of 1 ml/kg body-weight. Animals from both groups were sacrificed in batches at varying intervals up to 10 weeks. The livers were examined grossly and microscopically.

On gross examination livers from animals in group 2 show very little or no cirrhosis in contrast to those from group 1 where cirrhosis is apparent even in early phases of experiment. Microscopically in all animals of group 2 there is marked inhibition of fibroblastic growth and fibrogenesis in spite of continuation of significant centrilobular necrosis. In the latter areas there is collapse of the sinusoidal reticulum but new fibre formation and organization is greatly retarded or absent. Even after sixteen doses of CCl_4 there is no significant fibrogenesis. In animals of the high-protein group regular cirrhosis is present. Stains for acid mucopolysaccharides show no significant difference between the two groups.

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