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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Fourteenth meeting of The Nutrition Society (Fifty-first of the Scottish Group) was held in the Institute of Physiology, University of Glasgow, on Saturday, 8 February 1958, at 2 p.m., when the following papers were read:

The action of cortisone on protein metabolism at different levels of protein and energy intake.

By G. A. J. GOODLAD (BEIT MEMORIAL RESEARCH FELLOW) and H. N. MUNRO, *Department of Biochemistry, University of Glasgow*

Cortisone causes an increased excretion of nitrogen due to the breakdown of muscle protein and at the same time the protein content of the liver increases (Trémolières, Derache & Lowy, 1955). In an attempt to throw light on the mechanism of this action of cortisone on protein metabolism, we have studied the effects of cortisone on liver protein and ribonucleic acid phosphorus (RNAP), and on N balance for groups of female rats receiving diets varying in protein and energy content.

In control groups of rats, an increase in the energy content of the diet brought about an increase in liver protein and RNAP and a less negative N balance on diets of intermediate and high protein content. However, on a protein-free diet, increase in energy intake resulted in a fall in liver protein content. These results are similar to those previously obtained with male rats (Munro & Naismith, 1953).

Injection of 5 mg/day of cortisone acetate caused an increase in liver protein and RNAP content and an augmented excretion of N, at all levels of dietary protein and energy. The magnitude of the response of N balance to cortisone did not appear to be related to the energy content of the diet, the difference between the low- and high-energy groups at each protein level being only a reflection of the pattern observed with the control rats. There was, however, some evidence of a greater effect of cortisone on liver protein and RNA at the higher plane of energy intake. Thus the action of cortisone on protein metabolism seems primarily to be on the carcass, to which the liver changes are secondary.

These conclusions are being considered in relation to the wasting associated with the presence of a malignant tumour in the body. In this instance also, there is loss of carcass protein and accumulation of protein in the liver, accompanied by evidence of adrenocortical overactivity (Stewart & Begg, 1953).

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Dietary protein and the metabolism of ribonucleic acid in rat hepatoma. By H. N. MUNRO and C. M. CLARK, *Department of Biochemistry, University of Glasgow*

In a previous study (Clark, Naismith & Munro, 1957), we examined the uptake of ^{14}C-glycine and of ^{32}P by liver ribonucleic acid (RNA) of normal rats and found conditions under which the incorporation of these precursors was extensively influenced by the protein content of the diet. Rats fasting after a protein-containing meal had a much lower uptake of these labelled precursors than animals fasted after a protein-free diet, but uptake was rapidly restored by feeding protein. A considerable degree of sensitivity of liver RNA metabolism to the immediate supply of amino-acids was thus established.

It seemed of interest to know whether a tumour derived from liver cells would still exhibit the same fluctuations in RNA metabolism in response to changes in the dietary supply of protein. Experiments have accordingly been carried out on rats bearing a transmissible hepatoma implanted subcutaneously, and the response of the tumour and of the normal liver to dietary protein intake have been compared. Whereas the livers of the tumour-bearing animals showed the usual low isotope uptake when the rats were fasted after a meal of protein, the tumour cells did not do so. The hepatoma cells are thus less dependent on dietary conditions than are the parent liver cells. This is in accord with the finding that the protein and RNA content of the hepatoma cell, unlike the liver cell, are not affected by the feeding of a protein-free diet.

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Cod-liver oil as a source of vitamin E. By T. MOORE, I. M. SHARMAN and R. J. WARD, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

For many years cod-liver oil has been known to be capable of intensifying the effects of vitamin E deficiency in experimental and farm animals. Thus in rats which were given a basal diet deficient in vitamin E the inclusion of cod-liver oil caused brown discoloration, and peroxidation of the body fat (Dam, Granados & Prange, 1949). The introduction of cod-liver oil into the diet of calves may precipitate muscular dystrophy characteristic of avitaminosis E (Blaxter, Brown, Wood & MacDonald, 1953).

It was surprising, therefore, when Brown (1953) found by chemical analysis that cod-liver oil, and the liver oils of other fish, contained substantial amounts of α-tocopherol. He assumed that the action of cod-liver oil in precipitating signs of vitamin E deficiency was due to its high content of highly unsaturated fatty acids, rather than to the absence of the vitamin.

We have confirmed both that cod-liver oil is deleterious in inducing certain of the effects of avitaminosis E, as demonstrated in rats, and that it is nevertheless a good source of vitamin E. By chemical tests a specimen of cod-liver oil, of high

quality as used for medicinal purposes, was found to contain 10·2 mg α-tocopherol/100 g. With their diet containing 10% of this oil it may be calculated that our rats received at least 0·7 mg of α-tocopherol weekly from this source. This intake may be compared with a minimal requirement of about 0·3 mg necessary to prevent signs of deficiency when the diet contains lard instead of cod-liver oil. The effect of cod-liver oil in preventing or inducing the signs of avitaminosis E differed decidedly according to the particular sign which was under consideration. Thus cod-liver oil, presumably by virtue of its vitamin E content, gave complete protection, at least for several months, against brown discoloration of the uterus and degeneration of the testes. No protection was given, however, against brown discoloration of the body fat, haemolysis by dialuric acid, or post-mortem autolysis in the kidneys. In females which had received diets containing either cod-liver oil or lard for about 5 months striking contrasts were seen between the uterus and the fat deposits attached to this organ. With lard in the diet the uterus was brown, while the adjoining fat deposits were white. With cod-liver oil in the diet the fat was a deep brown, but the colour of the uterus was normal. It appears, therefore, that the results of the antagonism between vitamin E and highly unsaturated fatty acids, when both are supplied in the form of cod-liver oil, are widely divergent in different parts of the body.

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Some studies on the isolation of proteolytic bacteria from the rumen. By T. H. Blackburn and P. N. Hobson, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Some rumen bacteria have been found to degrade casein or gelatin during biochemical tests. Appleby (1955) specifically seeking proteolytic bacteria with a medium containing casein reported that *Bacillus licheniformis* was the chief proteolytic organism in the three sheep examined. The present experiments were designed to test for the presence in the rumen of bacteria capable of degrading proteins included in the diet, and preliminary results are given here.

Two sheep were fed a normal diet of hay and concentrate containing white-fish meal. An aqueous extract of the fish meal, with mineral-salts solution, was made the basis of media used. Medium 1 contained fish meal (2%); 2, fish meal plus yeast extract (0·5%); 3, fish meal plus glucose (0·3%); 4, fish meal plus glucose plus yeast extract. Reinforced clostridial medium (5) was also used to get an approximate idea of the total viable organisms in the samples. Initial dilutions were made in semi-solid media incubated under CO_2 for 24–48 h at 37°.

Although there were some variations with different samples the following results are typical. From sheep no. 416 medium 5 gave a mixed growth up to 10^{-9} dilution. Medium 1 gave slight growth to 10^{-6} dilution, the highest dilutions containing Gram-negative or variable rods. In medium 3 Gram-positive cocci predominated

in the highest dilutions (10^{-8}). Medium 4 gave a more varied growth with Gram-positive cocci, Gram-negative diplococci and coccobacilli and mainly Gram-negative, often granular, rods in 10^{-7} and 10^{-8} dilutions. From sheep no. 86, medium 1 gave growth up to 10^{-4} to 10^{-5} dilution, the highest dilutions containing mainly Gram-negative or variable rods. Medium 2 gave similar growth to 10^{-5} dilution, medium 3 mainly Gram-positive cocci at 10^{-8} dilution, and 4 a similar growth at 10^{-7} and 10^{-8} dilution. The substitution of casein for fish meal in media 2 or 4 gave mainly Gram-positive cocci in 10^{-5} and 10^{-9} dilutions of samples from sheep no. 86. From these media subcultures were made on to plates and proteolysis tested for by clearing of the medium and hydrolysis of protein precipitable by acid $HgCl_2$. Not all the organisms growing in the initial cultures were proteolytic but highly proteolytic representatives of the two groups, the Gram-positive cocci, whose growth was enhanced by glucose in the medium, and Gram-negative rods, have been isolated for further study.

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Fermentation of pentoses by suspensions of mixed rumen bacteria. By B. H. HOWARD (introduced by J. DAVIDSON), *Rowett Research Institute, Bucksburn, Aberdeenshire*

It is already known that bovine rumen liquor readily ferments D-xylose and L-arabinose to volatile fatty acids (V.F.A.), CO_2, and bacterial protein and polysaccharide (McNaught, 1951). Washed suspensions of mixed bovine rumen bacteria convert glucose or D-xylose into a starch-like polysaccharide (Robinson, Doetsch, Sirotnak & Shaw, 1955), and a similar transformation occurs during growth of the rumen organism *Bacteroides amylogenes* on glucose, D-xylose or L-arabinose (Doetsch, Howard, Mann & Oxford, 1957).

The action of sheep rumen liquor on pentoses has now been investigated, using liquor from a hay- and concentrate-fed sheep, after removing protozoa by centrifugation. Incubation with 0·5% (w/v) of the pentose was carried out at 38° under CO_2.

D-xylose and L-arabinose were completely decomposed under these conditions in 10 h. L-xylose was not decomposed at all, and D-arabinose was attacked slowly at first, and more slowly later, only 35% being used up in the 24 h experimental period. The rates of fermentation of D-lyxose and D-ribose increased during the experiment; finally 25 and 90% respectively had been decomposed. During the early stages of fermentation of D-xylose and L-arabinose about 30% of the sugar used was converted into V.F.A. and 30% into glucose in the bacterial cells. Higher proportions, 60 and 70% respectively, of the D-arabinose and D-lyxose fermented were converted into V.F.A., but no glucose was synthesized from either of these sugars. Conversion of D-ribose into glucose occurred only in the later stages of the fermentation, although V.F.A. were formed from the beginning. Lactic acid was produced only from D-ribose, and only near the end of the experimental period.

The ability of the bacterial suspensions to break down D-arabinose to V.F.A. but their inability to synthesize glucose from the intermediate metabolites is at present inexplicable. The relative ease of conversion into glucose of the different pentoses by the bacterial suspensions parallels very closely the ability of *Aspergillus flavus* to convert pentoses into a hexose intermediate in the biosynthesis of kojic acid (Arnstein & Bentley, 1956). The storage of glucosan by the bacteria while attacking readily fermentable sugars is similar to the behaviour of the rumen holotrich protozoa, and may be of value in keeping the rumen fermentation in a fairly steady state when food intake is intermittent (Oxford, 1955). The results obtained with D-ribose suggest that in vitro fermentations by rumen liquor extending beyond a few hours may not accurately reflect conditions in the rumen itself.

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The effect of dietary advice on the rate of weight gain during pregnancy.

By NAN R. TAGGART and A. M. THOMSON, *Obstetric Medicine Research Unit, Medical Research Council, Foresterhill, Aberdeen*

In primigravidae with uncomplicated pregnancies the average rate of weight gain is slightly over 1 lb./week from the 20th to the 30th week and slightly under 1 lb./week from the 30th to the 36th week; higher and lower rates of gain are associated, respectively, with an increased incidence of pre-eclampsia and of prematurity (Thomson & Billewicz, 1957). In a current clinical trial, primigravidae who gain weight at rates appreciably higher or lower than the above averages are seen by a dietitian who advises those gaining too much weight to take a low-calorie, high-protein diet, with restriction of sodium if oedema is present, and those gaining too little weight to increase calorie intake, emphasis being placed on frequent meals of moderate size. Each experimental subject is paired with a control primigravida of similar age, height, stage of gestation, social class and initial weight, who is not seen by a dietitian. Preliminary results presented here compare the first 304 pairs of experimental and control subjects, in terms of weight gained. Dietary advice has caused a considerable reduction in the number of patients with a high rate of gain but not in the number with a low rate of gain. Seventeen of the experimental patients had low average rates of gain because of dietary restriction imposed after an initially high weight gain.

Very few of the patients asked to restrict their diets were completely uncooperative. Those who failed appeared on the whole to be less intelligent and less well-educated than those who succeeded. Success usually required a sustained effort, which was often not maintained. It is more difficult to account for the failure to augment unduly low rates of gain in weight.

Numbers of experimental and control subjects (latter in parentheses) with various rates of weight gain

	Rate of gain, 30–36 weeks (lb./week)				
Rate of gain, 20–30 weeks (lb./week)	Low (<0·60)	Moderate (0·60—0·99)	Moderately high (1·00—1·39)	Very high (1·40 +)	All
Low (<0·80)	23 (24)	27 (27)	14 (8)	1 (6)	65 (65)
Moderate (0·80—1·19)	26 (22)	77 (46)	47 (21)	11 (18)	161 (107)
High (1·20 +)	10 (6)	28 (34)	28 (39)	12 (53)	78 (132)
All	59 (52)	132 (107)	89 (68)	24 (77)	304 (304)

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Changes in stored herring meal. 1. Nutritive value of the protein. By K. J. CARPENTER, *School of Agriculture, University of Cambridge*

Under certain storage conditions the protein of a food, such as dried milk, may lose nutritive value through reaction with carbohydrate (cf. Henry, Kon, Lea & White, 1948). Work with model systems suggests that similar results could occur when food protein is in contact with rapidly oxidizing fat (Tappel, 1955). This study was initiated with herring meal as a possible site for such reactions.

A 'control' batch (C) of herring meal (hot-air dried press-cake) was transported from the drier in a container with solid CO_2, then held under N_2 at $-20°$ until use. It contained 70% crude protein, 11% moisture and 11% petroleum-ether extract. A portion (S) was transferred for 71 days to storage in air at 25° to induce extensive oxidation of the lipids (Lea & Parr, 1958). Analysis for 'available lysine' (Carpenter, Ellinger, Munro & Rolfe, 1957) showed 5·03% in (C) and 4·83% in (S).

Two tests were then run with chicks receiving experimental diets *ad lib.* from their 10th to 20th day. Each herring meal was fed first to add 0·145% available lysine to a specifically lysine-deficient mixture based on sesame meal (Grau & Almquist, 1944); and then to add 0·29% available lysine to a more practical mixture of cereals and vitamin supplements containing 8% crude protein (Carpenter, Ellinger & Shrimpton, 1955).

			Feed eaten (g/chick)		Mean weight gain (g)	
Trial	Type of basal	Nos./treatment	(C)	(S)	(C)	(S)
1	Sesame (lysine-deficient)	8 groups of 4 each	212	209	92	93
2	Mixed cereals	6 groups of 3 each	275	275	103	103

(S) has apparently suffered no more serious reduction in value as a supplementary-protein source for rapid growth than that indicated by the 4% fall in available-lysine value, despite the extensive lipid changes. Nor have the changes affected the chicks' appetite at these low levels of feeding.

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Changes in stored herring meal. 2. Oxidation of the lipids. By C. H. LEA and L. J. PARR, *Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research*

It has been shown (Tappel, 1955) that in model systems containing highly unsaturated fat (linoleic acid or cod-liver oil), protein, and an oxidation catalyst (haemoglobin, haemin or cytochrome C) interaction of the protein with the oxidizing fat occurs, leading to the formation of yellow-brown products and subsequently of insoluble dark-brown copolymers of high nitrogen and oxygen content.

Almquist (1956) has recently reported a reduction in the percentage of fat extractable by solvents from stored fish meal, but gives no data on chemical changes.

Herring meal (Carpenter, 1958), part at its original (11·1%) moisture content and part after further drying in vacuo to 6·2%, was stored in air and in nitrogen at 10, 25 and 37°. The colour of the meal and the yield, peroxide value, iodine value and colour of the extracted oil were measured at intervals. Subsidiary experiments were run with the defatted meals.

A very rapid oxidation of the oil of the meals stored in air occurred, particularly during the first few weeks (see table). Oxidation was more extensive at the lower

				Extracted oil	
Moisture (%)	B.H.T. (%)	Days at 25°	Colour of the meal*	Iodine value	Colour ($E^{1\%}_{1\ cm}$ at 400 mμ)
6, 11	—	0	3·2	136	0·45
11	—	71	6·0	105	1·37
6	—	71	6·5	95	2·60
6	0·005†	71	5·5	129	0·85

* Lovibond units. † Plus 0·005% citric acid.

moisture content and at the lower storage temperatures, leading to darkening of the meals and to darkening, reduction in iodine value and solidification (polymerization) of the oils extracted from them.

Despite extensive oxidation of the oil $<5\%$ of the total lipid present became unextractable by solvents during the first 4 months. After 12 months the low-moisture meal had lost 6–8% and the high-moisture meal 11–15% of its chloroform–methanol-extractable lipid, corresponding approximately to 1 and 2% of the meal.

The anti-oxidant BHT (2, 6-di-tert. butyl-4-methylphenol) added to the meal at a concentration of 0·005% (together with 0·005% citric acid) considerably retarded oxidation of the oil.

Highly oxidized and polymerized oils are known to cause destruction of oxidizable nutrients, and to be toxic to laboratory animals when fed at high levels. At the low levels (<1%) used in the chick rations the highly oxidized oil of the fish meal produced no obvious adverse effects (Carpenter, 1958).

Further investigations involving longer periods of storage and stabilization by the use of anti-oxidants are in progress.

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The variations in the concentrations of copper and iron within and between the lobes of pig's liver. By J. CASSIDY and J. K. EVA, *Minsal Ltd, Northwich, Cheshire*

Four pigs' livers; 1, 2 and 3 from bacon weight pigs, and 4 from a pork pig of 120 lb. live weight, were taken for analysis. Pigs nos. 1 and 2 had been fattened from weaning on a diet containing 250 p.p.m. copper, the diet of pigs nos. 3 and 4 contained 500 p.p.m., both diets contained about 400 p.p.m. iron.

The livers were sectioned into the four lobes and the lobes further sectioned to give samples each weighing about 40 g. About thirty samples were obtained from each liver and the whole of each sample was analysed, copper and iron being determined.

The average results obtained are given in Table 1. The mean values are calculated from the known weight of sample and the concentrations of the elements found. Figures are expressed in p.p.m. of the fresh material.

Table 1. *Copper and iron concentrations in sections of liver*

		Liver 1			Liver 2			Liver 3			Liver 4		
Lobe	Element	No. of samples	Mean (p.p.m.)	Range (p.p.m.)	No. of samples	Mean (p.p.m.)	Range (p.p.m.)	No. of samples	Mean (p.p.m.)	Range (p.p.m.)	No. of samples	Mean (p.p.m.)	Range (p.p.m.)
R. lateral	Copper	5	73	68–78	5	114	84–144	10	440	324–603	5	15	14–16
	Iron		99	52–151		163	142–195		26	23–31		72	40–101
R. median	Copper	8	74	61–91	8	102	65–122	10	385	299–583	7	14	12–19
	Iron		97	58–162		207	155–255		25	22–29		90	47–115
L. median	Copper	8	72	61–88	8	90	51–118	10	371	261–437	7	11	8–18
	Iron		91	74–127		205	155–267		29	20–40		102	81–149
L. lateral	Copper	9	67	57–97	9	91	82–116	10	357	240–446	9	9	6–14
	Iron		113	72–144		152	108–177		28	23–36		60	35–90
Whole liver	Copper		71	57–97		97	51–144		389	240–603		13	6–19
	Iron		101	52–163		182	108–267		27	20–40		81	35–149

From the table it will be seen that there are considerable variations in the concentrations of copper and iron both within and between the lobes of pig's liver. From these results there is no evidence of a particular area in the liver which will give values identical to the whole liver values.

We submit that unless a sample representing the whole liver is analysed, copper and iron values found may be misleading.

If similar variations exist in the livers of other species, then liver-biopsy technique used in mineral-nutrition studies and diagnosis in cattle and sheep may be of limited value.

Relationship between the copper and iron concentrations in pigs' livers.

By J. CASSIDY and J. K. EVA, *Minsal Ltd, Northwich, Cheshire*

From preliminary studies undertaken in this laboratory on the copper and iron concentrations in pigs' livers, two important facts have emerged. The first, of which the results form the subject of a separate communication, is that owing to considerable variations in the concentrations of copper and iron both within and between the lobes of the liver, only a sample representative of the whole liver can give results which justify accurate interpretation; the second is that in experiments with pigs fed high levels of copper sulphate, there appears to be a relationship between the concentrations of copper and iron stored in the liver. The present paper deals with this second aspect.

The livers of twenty bacon pigs were obtained at slaughter, representing three groups of pigs which had been fed diets dissimilar only in their copper content. The diet of group 1 (seven pigs) contained 125 p.p.m. copper, that of the second group (six pigs) 250 p.p.m., and that of group 3 (seven pigs) 500 p.p.m. All diets contained about 400 p.p.m. iron.

To obtain samples representative of the whole liver, the following procedure was adopted. Each liver was sectioned into the four individual lobes and from each lobe several samples were taken from over the whole area of the lobe. These samples were combined to give a lobe sample of about 50 g weight, which was then analysed for copper and iron. From the data obtained, the concentrations of copper and iron in the whole liver were calculated.

The samples were prepared for analysis by the wet-ashing technique of Middleton & Stuckey (1954) and analysed for copper and iron by standard methods.

The average values and ranges of the individual results are given in Table 1. Figures are in p.p.m. of the fresh material.

Table 1. *Average copper and iron concentrations in the livers of pigs fattened on diets varying only in copper concentration*

	Copper (p.p.m.)		Iron (p.p.m.)	
Group	Average	Range	Average	Range
1 (125 p.p.m. copper)	39	11– 88	118	69–153
2 (250 p.p.m. copper)	154	23–354	92	58–134
3 (500 p.p.m. copper)	558	334–929	36	27– 47

From the average values of each group it will be seen that as the concentration of copper in the liver increases, corresponding to an increased copper intake from the diet, the iron concentration decreases, even though the intake of iron was the same for all groups. In other words, copper appeared to exert a depressing effect upon iron storage in the liver.

These findings indicate that an inverse relationship exists between the iron and copper concentrations in the liver.

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The effect of environmental temperature and level of feeding on food utilization by sheep. By D. G. ARMSTRONG, K. L. BLAXTER, N. McC. GRAHAM and F. W. WAINMAN, *Hannah Dairy Research Institute, Kirkhill, Ayr*

The energy exchange of two sheep, each closely clipped at weekly intervals so that its fleece was 1–2 mm long, was measured at seven environmental temperatures in the range 8–38° when given 600, 1200 or 1800 g dried grass daily. At environmental temperatures consonant with minimal heat production, the mean energy retentions of the sheep were +750 Cal./24 h (high level of feeding), +250 Cal./24 h (medium level of feeding) and −150 Cal./24 h (low level of feeding). Faecal and urinary losses of energy decreased as environmental temperature rose, hence the metabolizable energy of the food increased by 7 Cal./24 h/°C.

The environmental temperatures at which heat production was minimal were 39–40° for the low level of feeding, 33° for the medium level of feeding and 24–27° for the high level of feeding. Above these temperatures heat production increased exponentially with rectal temperature, the exponential coefficient of 0·075 ± 0·014 being equivalent to a doubling of metabolism for every 9° increase in rectal temperature and thus comparable to the Van't Hoff relationship for the reaction rate of isolated chemical systems. Below the temperature corresponding to minimal metabolism, heat production per unit body surface area increased at a constant rate irrespective of level of feeding. This rate was 80 Cal./24 h/m²/°C. corresponding approximately to 115 Cal./24 h/°C. for the sheep as a whole. At these low temperatures high levels of feeding resulted in abdominal distension, an increased surface and hence a greater actual heat loss compared with low levels of feeding.

Constant net energy values were not obtained because the thermoneutral zones at the different levels of feeding did not overlap. The net availability of the metabolizable energy of food was found to vary from 80 to 100% at low environmental temperatures down to 30–50% at very high environmental temperatures.

The heat increment of acetic acid partially neutralized with sodium hydroxide, in fasting sheep. By D. G. ARMSTRONG, K. L. BLAXTER and N. McC. GRAHAM, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Armstrong & Blaxter (1957) found that when acetic acid was given to fasting sheep as the sole source of energy its heat increment was 41%. This value greatly exceeded those found when propionic or butyric acids were given, a finding which might be the result of the marked acidosis that developed with acetic acid.

Two experiments were therefore carried out in which fasting sheep were given acetic acid buffered with sodium acetate, in order to prevent a fall in the pH of the rumen and in the CO_2-combining capacity of the blood. The mixture was infused into the rumen after an initial period of starvation during which dilute saline was given. The sheep were confined in a respiration apparatus (Blaxter, Graham & Rook, 1954) and oxygen consumption, carbon-dioxide and methane production and urinary nitrogen excretion were measured every 24 h. Blood samples were also taken.

The results are summarized in the table. They show that although the systemic acidosis was reduced by the presence of the sodium ion, the heat increment remained high—indeed, it was in excellent agreement with that obtained when acetic acid was given unbuffered. The high heat increment of acetic acid is thus not due to an acceleration of metabolism in response to an accumulation of hydrogen ions. Concentrations of steam-volatile fatty acids in rumen liquor and blood in the table refer to the total anion present as free acid and as salt. The markedly higher levels of steam-volatile fatty acids found in the rumen when the buffered acid was given were due to the sodium salts. The fall in blood sugar was less marked than that which occurred when acetic acid was given without the buffer.

	Acetic acid and sodium acetate		
	Exp. 1	Exp. 2	Acetic acid alone*
Calories given (Cal./24 h)	705	706	718
Calories metabolized (Cal./24 h)	565	608	657
Heat increment (Cal./24 h)	248	257	270
Heat increment/100 ml. acid metabolized	43·9	42·3	41·1
Rumen pH†	5·76	5·68	4·48
Steam-volatile fatty acids in rumen liquor (m-equiv./100 ml.)	10·39	11·26	8·86
Plasma CO_2-combining capacity of the blood (vol./100 ml.)	43·0	42·0	18·5
Blood sugar (mg/100 ml.)	34·1	46·7	28·0
Steam-volatile fatty acids in blood (m-equiv./100 ml.)	1·203	1·265	1·460

*The results are taken from the experiments reported by Armstrong & Blaxter (1957).
† All analyses of rumen liquor and blood samples refer to values obtained when the acid infusions had been given continuously for 72 h.

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The One Hundred and Seventeenth Meeting of The Nutrition Society was held at the Middlesex Hospital Medical School, Mortimer Street, London, W.1, on Saturday, 31 May 1958, *at* 10.15 *a.m., when the following papers were read:*

The mechanism of postoperative malabsorption syndrome leading to secondary protein malnutrition.

By Z. A. LEITNER, 52 *Welbeck Street, London, W.1*

A case in which postoperative malabsorption gave rise to the fully developed syndrome of protein malnutrition, believed to be the first of its kind on record, has already been described (Leitner, 1958*a*). The central role of the affected pancreas has been emphasized.

The occurrence of this syndrome has again been observed in a woman of 59 who had a pancreatico-duodenectomy in 1950. She was well after the operation at first, but 17 and again 29 months after pancreatico-duodenectomy she had to be admitted to hospital. On both occasions she was gravely ill, with extensive oedema, severe hypoproteinaemia, microcytic anaemia, osteoporosis, combined at her

first admittance with bilateral pleural effusion, and at her second with extensive exfoliative dermatitis. Since her recovery after the second relapse (February 1953) the patient carried on with a satisfactory life as a housewife (Leitner, 1958*b*).

Experience with these two patients indicates that pancreatico-duodenectomy (gastro-jejunostomy, cholecysto-gastrostomy with resection of the head of the pancreas) may affect the patient's prognosis in different ways, depending to a large extent on the preoperative condition of the remaining abdominal organs. Increased, or partially disordered gastro-intestinal motility is a natural sequel of this type of operation. The changed motility interferes to a variable extent with the adequate mixing of the chyme with the digestive juices, thus leading to a faulty absorption not only of the nutrients but also of the pancreas-stimulating hormones (secretin, pancrozymin, etc.). Increased fat content in the faeces after gastrectomy has been known for a long time (Taylor, Hudson, Dodds, Warner & Whitby, 1929). If, however, the remaining parts of the pancreas were already affected preoperatively, as in the above cases, the secretory function of the gland will be further diminished not only quantitatively, but probably also qualitatively. This faulty pancreatic secretion seems both to increase steatorrhoea and creatorrhoea, and also to hasten degeneration of the acinar tissue. As a result fibrosis of the pancreatic gland is aggravated. Additional sequels of pancreatic changes are increased hypoproteinaemia with reversion of the albumin-globulin ratio, and progressive fatty infiltration of the liver. A generalized avitaminotic state with skin and neurological changes, anaemia and osteoporosis follows.

Even the repeatedly fully developed syndrome of protein malnutrition may at times be reversible. Experience is insufficient at present to decide *a priori* the criteria upon which the reversibility of these pathological processes depends. Observations on severely malnourished babies, however, repeated on experimental animals (Véghelyi, Kemény, Pozsonyi & Sós, 1950; Véghelyi, Kemény & Sós, 1950), and the experience with the cases quoted above suggest that early energetic treatment with proteins (by mouth and by infusion) may be a successful first approach.

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Thyroid enlargement in schoolchildren. By D. E. HUGHES, K. RODGERS and DAGMAR C. WILSON, *Medical Research Council Unit for Cell Metabolism, Department of Biochemistry, University of Oxford*

A survey has been made of the incidence of thyroid enlargement amongst schoolchildren in north Oxfordshire, and the results compared with those obtained previously in the same area (Murray, Ryle, Simpson & Wilson, 1948). No significant change has been found in boys but there has been a marked increase in thyroid

enlargement in girls ($P<0{\cdot}001$; from 26·9% to 40·4%). The incidence in both surveys is higher in girls than in boys ($P<0{\cdot}001$; 40·4% in girls, 14·8% in boys) (Taylor, 1958). In one school (Hook Norton) the incidence of enlargement in both boys and girls was significantly higher than in the others ($0{\cdot}01>P>0{\cdot}001$ for boys; $P<0{\cdot}001$ for girls). The iodine level in milk from farms from the north Oxfordshire area was not significantly different from that in Essex and Wales. The cows' drinking water contained 18–117 μg I/l. in Essex compared to 2·2–2·9 μg/l. in Oxfordshire and 1·7–5·3 μg/l. in Wales. In the three areas milk obtained in July was markedly lower in iodine than milk obtained in November. This is probably due to supplement feeding in winter. The iodine content of the water supply to Hook Norton school was low (2·0 μg/l.) compared to the piped supply to most of the houses in the village (4·0–5·2 μg/l.), but was higher than that from local wells (0·6 μg/l.). The excretion of iodine in the urine was not found to be significantly different in representatives from Hook Norton and Oxford.

It is well established that thyroid enlargement results from low iodine intake (Stanbury, Brownell, Riggs, Perinetti, Itoiz & Del Castillo, 1954) or less frequently by interference with iodine metabolism by substances present in food (Astwood, 1949). It is not certain which of these factors is responsible for the continued high incidence of thyroid enlargement found in north Oxfordshire. The evidence suggests that the main cause is related to the relatively low iodine content and the hardness of the water which is typical of a predominately limestone area (Taylor, 1954). The results indicate that some iodine supplement such as iodized salt is necessary in this area (Astwood, 1949).

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The response of obese human subjects to isocaloric diets. By A. Kekwick and G. L. S. Pawan (introduced by E. C. Dodds), *Department of Medicine, The Middlesex Hospital Medical School, London, W.*1

Current theories of human obesity have for some time assumed that obesity is caused by overeating and treatment is therefore directed at reducing calorie intake below the theoretical energy requirements of the individual.

A predicted tissue-weight loss, corresponding on a calorie basis to the energy deficit, should then ensue. Experience has shown that all obese persons do not respond in this way, nor can this failure be satisfactorily explained in all cases by differences in body-water content or non-co-operation by the patient.

On 1000 Cal. diets with a fixed water and salt intake, it has been shown that obese patients lose weight most rapidly when the carbohydrate content of the diet is minimal and most rapidly on a high-fat diet (Kekwick & Pawan, 1953, 1956,

1957). These responses do not appear to be solely the result of alteration in body hydration nor are they explained by defective food absorption. Evidence will be presented which suggests that on isocaloric reducing diets, some aspects of metabolism in the obese differ from those of the non-obese and that the composition of these diets may induce metabolic changes resulting in marked differences in the rate and extent of weight loss. Furthermore, obese patients on 1000 Cal. diets excrete greater amounts of a 'fat-mobilizing material' in the urine on a high fat than on a high carbohydrate intake. Normal subjects excrete large amounts of this material on complete food deprivation (Chalmers, Kekwick, Pawan & Smith, 1958).

It would appear that a reappraisal of current theories of obesity is needed, with attention particularly focused on metabolic processes and accurate measurements of total energy output over prolonged periods of time.

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New process applied to meat preservation. By C. RADOUCO-THOMAS, R. ZENDER, C. LATASTE-DOROLLE, R. BUSSET and R. F. MOUTON (introduced by W. SCHWICK), *Laboratories of the Battelle Memorial Institute, Geneva, Switzerland.*

During storage, meat gradually disintegrates according to autolytic phenomena and microbial contamination. Autolysis in mutton, aseptically excised and stored, has been discussed previously (Zender, Lataste-Dorolle, Collet, Rowinski & Mouton, 1958). The present work shows that the ante-mortem administration of adrenalin (adequate dose and killing-time delay) is able to inhibit the autolytic process during aseptic storage (several weeks at 25° and 35°). This inhibition has been checked through various biochemical, microscopic, and structural tests carried out on different laboratory animals (rats, rabbits) and slaughter-house animals (pigs, cattle, sheep).

The comparative study (Radouco-Thomas, Zender, Lataste-Dorolle, Collet & Mouton, 1958) of adrenalized and non-adrenalized muscles shows:

(a) In the controls, a first phase of predominantly exudative phenomena characterized by a drop of pH (7·2–5·5), a rigor contrast-phase appearance, much sarcoplasma exudate (of high protein level and K/Na∼10). A second phase of predominantly autolytic disintegration phenomena characterized by metabolic, structural, and organoleptic modifications leading to a gradual disruption of the myofibril framework.

(b) In the adrenalized samples, inhibition of the autolytic process, in the first phase, the 'glucidic level' is depressed, the drop of pH and the exudation strongly inhibited, and the band-pattern appears mainly in alternate shortening–stretching. In the second phase, during storage, metabolic and structural disintegration modulus appears stopped or reduced, the muscles remain turgescent, fibres and myofibrils

appear intact; the organoleptic characteristics seem to be stabilized and moreover improved, especially the appreciable tenderness ($P=1\%$, i.e. 79·4% of the population).

The mechanism of the adrenalin action is discussed.

Adrenalin cannot be considered as an 'additive product', for it is normally produced and quickly destroyed by the organism.

The effects of adrenalin and like compounds on other animals and vegetable foods are being studied further.

Practical use of the data obtained seems already possible. The process described being harmless, producing better meat resistant to long storage and various temperatures, even normal and tropical, could allow a more efficient meat distribution.

The 'adrenalized inhibition process' should, however, be completed by various microbial inhibition techniques, such as antibiotics, ionizing radiations, etc., which problems are dealt with in ulterior works (Radouco-Thomas, Lataste-Dorolle, Zender, Busset, Mouton & Bellamy, 1958; Zender, Lataste-Dorolle, Collet, Rowinski, Radouco-Thomas & Mouton, 1958).

This investigation was made under the sponsorship of the General Electric Research Laboratories, Schenectady, New York.

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A numerical index of protein nutrition and function in human adults.

By R. L. WORRALL, 31 *Braeside Avenue, Sevenoaks, Kent*

Functional tests are necessary in assessing the protein nutrition of human adults. In contrast to nutrition studies of domestic animals, where accumulation of a maximum weight of skeletal-muscle protein in a minimum period of time is an accepted criterion, the protein nutrition of human adults must be estimated with reference to protein function under standard conditions of stress.

The normal protein content of the adult human body is 15–20% of body-weight, but this figure is significant only with reference to the differential distribution and functional pattern of protein in various tissues.

The functions of protein in various tissues are directed in general towards homeostasis, and muscular effort is necessary to preserve homeostasis under stress. This fact may be used to define an index of protein nutrition and function in human adults under the standard stress of gravity while sitting and then standing. The index is calculated as follows: (1) Count b, the pulse rate/0·5 min of a rested individual

in the sitting position (not in an armchair, and not soon after a meal or a bath). (2) Let the individual stand up and remain standing for 0·5 min, without counting or talking, in order to let the pulse rate become constant after the effort of standing up. (3) Count *a*, the pulse rate/0·5 min while the individual remains standing. Then

$$a^2 - b^2 = (a + b)(a - b) = R,$$

where R is an index of a neuromuscular reaction of body protein to standard stress.

Relatively low values of R, below 250, indicate more or less satisfactory protein nutrition with reference to homeostatic function. In the absence of disease, the value of the index has been found to range from $32^2 - 30^2 = 124$ for a female ballet dancer aged 24 years, to $43^2 - 38^2 = 405$ for an 80-year-old woman showing marked muscular wasting.

If the calculation is applied to cases of disease, the index becomes a numerical expression of a patient's general condition, rising in value with deterioration of that condition.

The effect of light on fattening pigs. By R. BRAUDE and K. G. MITCHELL, *National Institute for Research in Dairying, Shinfield, Reading*, and P. FINN-KELSEY and V. M. OWEN, *Electrical Research Association, Shinfield, Reading*

Four cabins were used in which the temperature (62–68°F.) and relative humidity (72–78%) were kept constant throughout the test period. Each of the cabins had different artificial-light treatment (see table). Litter-mate pigs 9 weeks old and of similar weight were distributed at random between the four cabins, each cabin taking four pigs. Meal was given dry *ad lib.* and unrestricted water was available.

Effect of light on fattening pigs

(Initial live weight 37–38 lb.)

	Cabin 1 24 h darkness	Cabin 2 14 h light + 10 h darkness	Cabin 3 10 h light + 10 h darkness (20 h day)	Cabin 4 24 h light
	9 to 19 weeks (eight pigs per treatment)			
Final weight (lb.)	129·0	124·2	125·9	127·8
Daily gain (lb.)	1·3	1·2	1·3	1·3
Efficiency of food utilization (lb. meal/lb. gain)	2·8	2·9	2·8	2·9
	9 weeks to bacon weight (four pigs per treatment)			
Final weight (lb.)	205·2	201·2	203·5	202·0
Daily gain (lb.)	1·4	1·3	1·3	1·3
Efficiency of food utilization (lb. meal/lb. gain)	3·3	3·4	3·4	3·4

The first replicate was on experiment for 10 weeks, the pigs being slaughtered as porkers (average of 126 lb. live weight), while the second replicate was continued to bacon weight (average of 203 lb. live weight).

The small number of animals involved in the test does not allow final conclusions to be drawn, but the data in the table indicate that the different light treatments did not influence the performance of the pigs. Pigs kept continuously in the dark

or continuously in the light were in no way adversely affected when compared with pigs kept in normal daylight hours (14 h light + 10 h darkness). The attempt to improve performance by keeping pigs under a 20 h day régime was unsuccessful. The time spent at the trough by the pigs in cabins 1 and 2 was continuously recorded. The pattern of food consumption appeared to be similar in both groups.

The amino-acid standards for calculating chemical score. By A. E. BENDER, *Research Department, Bovril Ltd, 148 Old Street, London, E.C.1*

Chemical score (C.S.) is useful in forecasting the nutritive value of a protein from its chemical analysis. The amino-acids present are compared with those of egg protein and the amount of the limiting amino-acid, expressed as a percentage of the same amino-acid in egg protein is the C.S. (Block & Mitchell, 1946–7; Bender, 1954).

A critical point is whether egg protein is perfect. Its biological value (B.V.) is approximately 100, and cannot be significantly improved by supplementation. But certain of the amino-acids could be present in amounts greater than required, when C.S., in instances where any of these were limiting, would be too low. The following experiments showed that some amino-acids could be reduced in quantity without affecting B.V. so lowering the target figures for calculating C.S.

(1) Egg protein was diluted by the addition of 15% of non-essential amino-acid mixture (N.E.A.A.): N.P.U. (Miller & Bender, 1955) 99. (2) Egg protein diluted 30%: N.P.U. 82. (3) An amino-acid mixture was prepared to simulate egg protein according to the most thorough analysis available (Rutgers University Bureau of Biological Research, 1950): N.P.U. 90. (4) Mixture (3) diluted 15% with N.E.A.A.: N.P.U. 93. (5) Mixtures of amino-acids were made as in (3), but each amino-acid in turn was halved in amount. If the amino-acids of (3) had been 100% of the rat requirements each of these mixtures should have N.P.U. 50. Where results are higher than 50 then mixture (3) contained more than 100% of that particular amino-acid. Biological determinations showed lysine 100%, histidine 120%, tryptophan 160%, phenylalanine 150%, cystine + methionine 100%, threonine 120%, valine 140%, leucine + isoleucine 100%.

From these figures the amino-acid requirements of the rat were calculated in terms of percentage composition of the ideal protein (the figures in parentheses are those used by Block & Mitchell in their calculations of C.S.): histidine 1·8% (2·1), lysine 5·2 (7·2), tryptophan 0·7 (1·5), phenylalanine 3·8 (6·3), cystine 2·0 (2·1), methionine 2·7 (4·1), threonine 4·1 (4·9), leucine 7·5 (9·2), isoleucine 4·3 (8·0), valine 5·0 (7·3); N.P.U. of this mixture 92. The values are close to mixture (4).

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The relationship between amino-acid composition of bread protein and its biological value. By J. A. PALGRAVE (introduced by A. E. BENDER), *Research Department, Bovril Ltd, 148 Old Street, London, E.C.1*

Samples of commercial white bread have been analysed for their amino-acid content by ion-exchange chromatography (Moore & Stein, 1951) and found to agree fairly closely with similar analyses of small laboratory-baked loaves by McDermott & Pace (1957).

Using the improved amino-acid standards reported in the previous communication it is possible to calculate the successive bottlenecks imposed by the various limiting amino-acids. These were found to be as follows, the figures representing the chemical score: lysine 29, threonine 66, valine 82, cystine and methionine 87, isoleucine 89, leucine 94, histidine, tryptophan, phenylalanine 100.

The suggestion was tested biologically by feeding bread supplemented with amino-acids and measuring the biological value (N.P.U. ÷ digestibility) by the carcass-nitrogen method (Miller & Bender, 1955). B.V. of bread alone 50, + lysine 61, + lysine + threonine 75, + lysine + threonine + methionine 86. The order of bottlenecks is thus lysine, threonine, methionine, confirming the order suggested by the chemical method for the first two amino-acids. The finding for threonine confirms the results of Sure (1952). All other amino-acids were tried but it was not possible to improve the B.V. beyond 86. It is suggested that the failure of the chemical and biological methods to agree in the placing of the sulphur amino-acids or valine lies in the difficulty of distinguishing biologically between 82 and 87.

Thus we have serially supplemented bread protein with its required amino-acids and achieved a final mixture with a B.V. reasonably close to a perfect protein.

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Protein malnutrition—a note on nomenclature. By B. S. PLATT, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, London, N.W.7*

More than fifty names, not including many vernacular ones, have been given to syndromes, the main and common element in which is an absolute or relative insufficiency of protein in the body, and usually in the diet of the affected subject. If the term malnutrition is used to include under- and over-feeding, two main types of protein malnutrition can be distinguished (i) marasmus; and (ii) kwashiorkor, or starchy food dystrophy, or third-degree malnutrition. These types overlap, hence the clumsy term marasmic kwashiorkor.

The marasmic animal or child may be more deficient in protein than the subject with kwashiorkor. The essential difference between the two types is that in the

second the diet provides more calories derived from carbohydrate; more from fat instead of carbohydrate would be beneficial. Marasmus and pre-kwashiorkor in my experience can occur in an infant fed entirely on mother's milk; the 'sugar baby' is characteristic of the second type. Maternal 'loving tender care' (it often amounts to 'forced feeding', as for the production of pâté de foie gras), is an element in the development of the second type which has not been recognized.

Malnutrition has often an important role in the pathogenesis of many diseases. The problem of nomenclature is therefore complicated by the fact the protein malnutrition may be (a) associated with deficiencies of other food factors also recognized in terms like 'sindrome policarencial' and may be found together with other classical dietary deficiency diseases, including possibly rickets; (b) conditioned by concomitant alterations in endocrine-gland activity (as in pseudo-hypophysectomy, and the production of fatty liver in insulin insufficiency); and (c) exacerbated by infectious diseases which precipitate death and complicate the pathology.

Nomenclature is important in view of the growing tendency to equate the term kwashiorkor with protein malnutrition; the term thus loses its acclaimed merit of 'neutrality' and there is a risk that much protein malnutrition (i.e. of the marasmic type) might be overlooked. Furthermore, overfeeding or underfeeding of low-protein, high-carbohydrate diets, possibly also imbalanced in respect of other constituents—fat, vitamins and minerals—is almost universal and the chronic effects of such malnutrition may be reflected in the prevalence of many disorders, e.g. of adolescence and reproduction in women. The word malnutrition should be included in any name.

A complete text with a bibliography of twenty-four references, omitted owing to lack of space, is available on request to the author.

The effects on pigs of a low-protein diet with and without additional carbohydrate. By C. R. C. HEARD, B. S. PLATT and R. J. C. STEWART. *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, London, N.W.7*

Pathological changes in piglets fed on a 'Gambia-type' diet have been reported in previous communications to this Society. Striking differences in the response of young animals have now been obtained by supplementing this diet with additional carbohydrate (starch–glucose mixture). The results given in the table are for nine animals (three in each group) and the figures are the mean values at death, since these did not differ for animals killed between 83 and 223 days of age.

The results show that pigs fed on a low-protein diet develop a syndrome resembling marasmus in children; the assiduous feeding of extra calories as carbohydrate, far from ameliorating the condition, precipitates a form of protein malnutrition resembling kwashiorkor.

	Commercial diet	5% protein diet (160 g/day)	5% protein diet (160 g/day) +carbohydrate (100 g/day)
Protein intake (N × 6·25) (g/day)	70 (at 5 kg)	8·8	8·9
Calorie intake (Cal./day)	1000 (at 5 kg)	540	920
Weight (kg), range and (mean)	62–120	4·9–5·3 (5·1)	4·4–5·6 (5·0)
Length of radius, increase from 14 days (cm)	7 (at 125 days)	1·3	0·8
Plasma alkaline phosphatase*	14	6·8	4·2
Skin fat : fat-free dry weight	7·7	2·6	5·7
Skin water: fat-free dry weight	2·7	6·6	9·3
Pancreas: volume of individual islet cells as percentage of normal cell volume	100	61	39†
Plasma amylase ‡	900	530	790
Haemoglobin (g/100 ml.)	12	9	7
Total plasma protein (g/100 ml.)	8·3	6·4	4·4
Serum albumin (g/100 ml.)	3·0	2·3	0·7
Liver fat (histological) §	0	0–1+	3+
Liver glycogen (histological) §	0–1+	2+	3+
Total urinary N : N intake, %	—	55	41
Urinary urea N : total urinary N, %	—	81	63

* King-Armstrong units/100 ml.

† One pig only (195 days); recent more acute experiment gave at 61 days no difference between diets of 5% protein and 5% protein + carbohydrate.

‡ Somogyi units/100 ml.

§ Some livers were also analysed chemically, confirming histological findings: 3+ approximates to 3% wet weight for fat, and 7% wet weight for glycogen.

Ascorbic-acid requirements of the dog. By D. J. NAISMITH (introduced by B. S. PLATT), *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, London, N.W.7*

It is generally assumed that the dog does not require ascorbic acid in its diet, but statements in the literature are conflicting (Innes, 1931; Lacroix, Park & Adler, 1942). Numerous reports have appeared on scurvy-like conditions in dogs, which have responded to treatment with ascorbic acid.

The purpose of the present experiments was to determine whether, during the first days of life, the dog is independent of an exogenous supply of ascorbic acid, particularly in view of the relatively high level found in bitch milk (4·4 mg/100 ml.) and to note any changes in the utilization of ascorbic acid by the bitch during lactation.

Three litters were used. One half of each was left as a control group with the bitch, 1 day (two litters) or 8 days (one litter) after birth. To the others, a synthetic diet, approximating to the composition of bitch milk, was fed, containing casein 5·0, egg albumin 2·5, butter 9·6%, a salt mixture, a vitamin mixture excluding ascorbic acid, and either lactose or sucrose 3·0%. In addition, one animal in each of the two experimental groups received 4 mg ascorbic acid daily.

Over periods of 3 or 7 weeks, blood samples from all animals, including the bitches, were assayed for ascorbic acid by the method of Roe & Kuether (1943).

The average values for mother-fed controls decreased regularly with time (2nd week 1·34 mg/100 ml.; 7th week 1·06 mg/100 ml.) There was no significant difference

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between the levels of the mother-fed dogs and those on ascorbic acid-free diets (averages 1·48 and 1·68 mg/100 ml. respectively). Neither the substitution of sucrose for lactose in the diet (sucrose 1·68, lactose 1·69 mg/100 ml.), nor the addition to it of ascorbic acid had any effect (with ascorbic acid 1·36, without 1·64 mg/100 ml.). The blood concentration in the pregnant bitches was approximately the same as that of the 7-week-old pups (average 1·14 mg/100 ml.). Immediately after parturition the values fell rapidly, in one case, after 3 weeks, being reduced to 67% of the original level.

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The nutritive value of meat dehydrated by the vacuum oil technique.

By P. L. PELLETT, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, London, N.W.*7

A vacuum oil process for the dehydration of meat has been recently described by Vere-Jones (1957) in New Zealand. The process was demonstrated in this country to the Biochemical Society (Platt, 1945) and also to this Society (Platt, Heard & Pellett, 1958).

In the present investigation minced beef, steak and liver were dehydrated in arachis oil at 70°. The net protein utilization (N.P.U.) was determined by Mr D. S. Miller, using the shortened method of Miller & Bender (1955), before and after processing. Riboflavin and nicotinic acid were assayed by microbiological methods and the thiamine by the thiochrome technique. Assays were also performed upon meat samples which had been dehydrated after pre-freezing, since it was found by Vere-Jones (1957) that the final product was considerably improved by this modification; our experience confirms this. The results obtained are shown in the table.

Vitamin content (μg/g on the fat-free dry-weight basis) and N.P.U. *values for vacuum–oil dehydrated meat*

Sample	Riboflavin	Nicotinic acid	Thiamine	N.P.U.
Fresh steak	9·1	193	1·8	75
Dehydrated steak	8·5	193	1·7	Not done
Pre-frozen dehydrated steak	7·9	179	1·6	75
Fresh minced beef	9·6	163	1·6	58±1*
Dehydrated minced beef	7·3	120	1·2	55±0
Pre-frozen dehydrated minced beef	7·6	127	1·2	57±2
Fresh ox liver	94	487	9·0	—
Pre-frozen dehydrated ox liver	107	461	8·4	—

* Acetone-dried sample.

The losses in B vitamins were quite small for the steak and liver but somewhat greater for the mince. Since the percentage loss of the heat-stable vitamin, nicotinic acid, was similar to the losses of the more heat-labile riboflavin and thiamine, it was concluded that the losses were probably due to exudation of meat liquors during

drying. Greater losses of B vitamins occurred upon dehydration of the pre-frozen than of the fresh steak.

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Nutritive value of wheat from the Rothamsted Broadbalk field. By D. S. MILLER and I. S. DEMA, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, London, N.W.7*

It has been suggested that wheat grown with organic manure is of greater nutritive value than that grown with artificial fertilizers (Milton, 1956) but little scientific work (Harris, 1934) has been done to test this hypothesis. The Broadbalk experiment at Rothamsted (Russell & Watson, 1940) presents a unique opportunity for such a study because of the range of fertilizer treatments which have been continued for more than 100 years.

Wholemeal flours were prepared from samples of wheat taken from three of the plots (2B dung; 3 no manure; 7 artificials) and examined for nutritive value and a commercial stone-ground wholemeal flour was used as a control. The table provides the data obtained.

	Whole-wheat meals from			
	Plot 2B (dung)	Plot 3 (no manure)	Plot 7 (artificials)	Commercial source
P (%)	0·48	0·43	0·46	0·41
Ca (%)	0·037	0·047	0·044	0·029
Mg (%)	0·14	0·14	0·14	0·16
K (%)	0·43	0·40	0·44	0·38
Na (%)	0·004	0·004	0·004	0·003
Mn (μg/g)	35	40	70	50
Thiamine (μg/g)	2·66	2·78	3·03	—
Riboflavin (μg/g)	1·80	1·18	1·53	—
Nicotinic acid (μg/g)	36·9	34·9	43·6	—
Fat (%)	0·7	0·9	0·9	—
Crude protein (%)	15·2	13·6	14·4	14·7
Net protein utilization*:				
Alone	32·4	39·5	37·8	35·4
With 0·6% DL-lysine	39·0	44·0	43·0	—
Net dietary value (protein)†	4·92	5·37	5·44	5·20

* By the method of Miller & Bender (1955).
† Net protein utilization × % crude protein.

In view of the similarity of these figures, successive generations of rats were fed on a diet of wholemeal flour, $CaCO_3$ 1%, NaCl 1%, and 800 i.u. vitamin A per week. Rats on the commercial flour failed to breed; those on the Rothamsted wheats did breed but there were no differences between the reproductive performance of the animals on wheats from the various plots, the yields from which were different.

We would like to thank Sir William Ogg for providing the samples of wheat, Dr R. G. Warren for the mineral analysis, Dr P. L. Pellett for the vitamin analysis, and Miss H. G. Sheppard for much of the animal work.

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The effect of heat treatment on the net protein utilization (N.P.U.) of [^{3}H] lysine–labelled pork. By I. S. DEMA, J. DONE, D. S. MILLER and P. R. PAYNE, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, London, N.W.7*

Acetone-dried pork which had been labelled (Done & Payne, 1958) with a specific activity of $3{\cdot}74 \times 10^{-4}$ μC/mg was moistened with an equal weight of water and heated in closed jars for 24 h at 110°: 'available' lysine was determined by the method of Carpenter & Ellinger (1955). The N.P.U. of treated and untreated materials was determined by the method of Miller & Bender (1955) and concurrently urine and faeces were collected for tritium analysis. The results are presented in the table.

		Digestibility		Biological value		N.P.U.	
	'Available lysine'	By N	By ^{3}H	By N	By ^{3}H	By N	By ^{3}H
Control pork	9·1	88	90	80	81	70	73
Heated pork	8·2	72	66	49	71	35	47

The biological data calculated from tritium activity are 'apparent'; 80% of the tritium was recovered in carcass, faeces and urine. The two methods demonstrate a severe impairment of N.P.U. Most of this reduction in the case of ^{3}H is accounted for by a lowered digestibility whereas the reduction of the figure for nitrogen is due largely to lowered biological value, although both effects are apparent in both cases. Methionine was the limiting amino-acid even after heat treatment (heated pork + methionine: N.P.U. = 60).

In another experiment, a solution molar with respect to labelled DL-lysine hydrochloride and glucose was heated for 24 h at 110°. A high molecular weight fraction was precipitated with 90% ethanol and had an activity equivalent to 32% lysine, and an elemental analysis of C = 54·1%; H = 6·1%; O = 32·6%; N = 7·2%. The material, as measured by radioactivity, was not digested by rats and addition to a lysine-deficient diet gave no improvement in N.P.U.

We are indebted to Dr K. J. Carpenter for the determination of 'available' lysine.

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Some dietary differences among manual workers' families associated with variations in income. By W. L. READMAN, *Economic Advice and Food Consumption Division*, and ELIZABETH ANNE DRURY, *Scientific Adviser's Division (Food), Ministry of Agriculture, Fisheries and Food, London*

1. The relation of income to some food habits of manual workers' families has been assessed by subsamples from the National Food Survey households of 1954, 1955 and 1956. Each family contained a male adult worker, a non-earning woman, and up to four children under 15. The childless couples were further limited to adults under 55.

2. For families of constant size a 10% increase in declared family income was associated with increases of 2·3% in the price/Cal., 0·9% in the energy value of the food entering the household and 3·2% in food expenditure; but energy requirements were virtually constant for the four income groups distinguished.

3. Comparisons between family sizes are less easy. The estimated energy requirements of childless couples were only 75% of the calorie value of food obtained, increasing with family size up to 90% (see table). Errors in the estimation of energy requirements and the calorie equivalents of food cannot vary greatly with the size and therefore do not account for these differences. If, as seems probable, actual calorie intakes of these families are near their energy requirements, then the remainder, estimated to vary from 1720 Cal./day/childless couple to 1280 Cal./family with four children, was just not eaten (at least by humans).

4. Although with increased incomes rather more calories were obtained at higher prices, the higher-income families, enjoying greater freedom of choice, showed virtually no preference for foods richer in protein or calcium. This is shown in the last line of the table, which expresses the results isocalorically. In the absence of such a preference it is probable that the food actually eaten contained these nutrients in proportions similar to those in the food obtained for consumption, and not varying with income. Thus the concentration of protein and calcium in the diet of manual workers' families is unlikely to be materially affected by changes in their income alone.

Family type (defined in para. 1)	No. of families in sample	Recommended allowance (g/1000 Cal.)		Food obtained for consumption (g/1000 Cal.)				Calorie requirement as a percentage of energy value of food obtained for consumption
				Protein				
		Protein	Calcium	Vegetable	Animal	Total	Calcium	
Childless couple	248	28·0	0·322	12·6	16·6	29·2	0·376	74·8
One child	869	29·5	0·391	12·9	16·2	29·0	0·402	80·9
Two children	835	30·5	0·433	12·9	15·7	28·7	0·414	85·4
Three children	698	31·3	0·458	13·6	15·0	28·6	0·415	88·5
Four children	328	31·8	0·470	14·1	14·3	28·4	0·407	90·3
Effect of 10% increase in family income		+0·006 (S.E.: 0·006)	−0·0009 (S.E.: 0·0003)	−0·06 (S.E.: 0·02)	+0·17 (S.E.: 0·03)	+0·10 (S.E.: 0·03)	+0·0002 (S.E.: 0·0008)	−0·7 (S.E.: 0·2)

The effect of the level of roughage in the rearing diet on the ability of adult cattle to utilize roughage. By C. C. BALCH, R. C. CAMPLING, V. W. JOHNSON and JILL ROY, *National Institute for Research in Dairying, Shinfield, Reading*

An experiment with five pairs of monozygous twin heifers began when the animals

were weaned at 14–19 weeks. One member of each pair was allocated at random to a high-roughage diet consisting of hay *ad lib.* and 2 lb. daily of a concentrate mixture containing oats (7 parts) and groundnut cake (1 part), with additional minerals and vitamins A and D. The twin of this animal received only 40% of the hay consumed by her sister and sufficient of the concentrates to ensure that each twin maintained a similar rate of growth and body size. Records of live-weight changes and skeletal measurements show that this object was achieved and it may be assumed, therefore, that any effects due to different rates of growth, or to different size, were eliminated.

A series of digestibility trials was conducted in the last few months before the birth of the first calf. The digestive efficiency of each twin was measured first with the diet on which she had been reared, secondly with the diet on which her sister was reared, and thirdly with a diet consisting entirely of hay. Both twins received the same amount of each diet. As exemplified by the results of the third trial (Table 1) there was no evidence that the type of diet given during the rearing period had any effect on the digestive efficiency of the adult.

After calving, each sister is receiving equal amounts of concentrates and hay *ad lib.* and observations are being continued through the first lactation; there is as yet little evidence of any consistent difference in either milk yield or appetite for roughages between animals reared on the two diets. Thus while it is not impossible that rearing could influence the utilization of the digestible nutrients, these further results suggest that under our experimental conditions the ability of the heifers to utilize roughages, was not affected by the diet during the rearing period.

Table 1. *Digestibility coefficients for a diet of hay alone in heifers reared on diets low or high in hay*

		Mean digestibility coefficient (%)					
Diet during rearing period	No. of heifers	Dry matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Ash
Low hay	5	58·8	53·4	28·0	61·5	63·6	38·4
High hay	5	58·8	52·5	27·7	61·6	63·0	39·6
Standard error of difference between means		±0·92	±1·43	±2·55	±0·81	±1·31	±1·05

Raw meat and carnivores: the effects of feeding ox heart to kittens. By J. P. Greaves, Patricia P. Scott and M. G. Scott, *Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.*1

We have previously shown that cats have a high requirement for protein. Searching for a standard by which to judge the value of different protein mixtures, kittens were fed on raw ox heart: an easily obtained 'natural' diet, readily consumed, rich in native protein, and probably having a reasonable constant composition.

In four experiments twenty-two kittens reared on our stock diet have been fed on heart, commencing at 10–14 weeks of age. After about 6 weeks on the heart diet, the following symptoms developed: incoordinate gait, particularly weakness

in the hind limbs; extreme nervousness; sensitivity to handling and sudden noises; a tendency to sit or lie rather than to take exercise. X-rays showed rarefaction of the skeleton, especially the vertebral column, pelvis and scapulae; spontaneous fractures occurred in some cases. Histological examination showed that the skeletal changes were not those of classical rickets since endochondrial ossification was proceeding almost normally. The changes resembled osteomalacia, bones showing hyperaemia and increased marrow and Haversian spaces, numerous osteoclasts and thin fenestrated layers of cortical bone. The thyroids were hyperaemic and hyperplastic with very little colloid, and the small intestine showed elongation of villi with sloughing of the epithelium at their distal extremities. Until the onset of symptoms, the growth of the animals was excellent and their condition good, the fur thick and lustrous, eyes bright and clear. Their general condition and appetite remained good, even after the cats had become partially paralysed. Heart is known to be low in calcium (8 mg/100 g wet weight) and to have a calcium : phosphorus ratio of about 1 : 20. There seems no reason to suspect a deficiency of vitamin D, but the vitamin A levels have been reported to be low. Iodine may also be deficient. Accordingly, some kittens are being supplemented variously with calcium gluconate, vitamins A and D and iodine. Those which received calcium gluconate showed no symptoms after 12 weeks on the diet; however, their thyroids were still abnormal.

Similar experiences are found in the literature with animals fed predominantly on flesh diets (Korenchevsky, 1922) and it appears that zoo lions which live almost entirely on muscle meat show similar symptoms (Jones, O., personal communication). Experiments are continuing on this problem.

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The effect of hexoestrol on the nucleic-acid content of the anterior pituitary gland of yearling male sheep. By E. M. MARTIN and G. E. LAMMING, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough*

Oestrogenic hormones are known to increase the growth rate of fattening ruminants. Several theories based on stimulation of anterior pituitary gland activity have been proposed to account for this effect. Proof of such a stimulation is hampered by the lack of satisfactory blood assays of pituitary-gland hormones. However, increase in hormone secretion should be accompanied by increased protein synthesis. Since the ribonucleic-acid (RNA) content of secreting tissue is thought to indicate its protein synthetic rate, a comparison of the nucleic-acid contents of anterior pituitary glands of hexoestrol-treated and control sheep was made.

One group of sheep was implanted subcutaneously with 15 mg hexoestrol and a second group of the same initial live weight left as untreated control. Ninety to a hundred days later animals were slaughtered and their anterior pituitary glands dried, weighed and analysed. Ribonucleic acid was determined by the method of

Hurlbert, Schmitz, Brumm & Potter (1954) and deoxyribonucleic acid (DNA) by the method of Burton (1956).

The results (Table 1) suggest that in animals where hexoestrol implantation caused an appreciable increase in growth rate (Exp. 1) their anterior pituitary glands show an increase in cell numbers (total DNA) a marked increase in cell size (decreased DNA/unit weight) and a significant increase in protein output (total RNA). In Exp. 2, where no extra growth due to treatment was achieved, there is no increase in cell numbers, some increase in cell size and a small increase in protein output of the gland.

In animals from Exp. 1 there was a significant linear relationship between growth rate and the total RNA content of the gland, and it is inferred that the effect of oestrogens on growth rate is mediated primarily through the anterior pituitary gland.

Table 1. *The effect of hexoestrol implantation on the nucleic-acid content of the anterior pituitary gland of sheep*

	Exp. 1		Exp. 2	
	Treated	Control	Treated	Control
No. of animals	6	6	6	6
Percentage growth rate†	22·6 ± 1·7	14·2 ± 1·4**	12·8 ± 3·1	14·4 ± 2·9
Anterior pituitary gland analysis:				
Dry weight	228 ±17·7	118 ± 7·5***	150 ± 6·3	127 ± 2·9
RNA-phosphorus:				
Total (μg)	450 ±38	240 ±17***	319 ±12	262 ±19*
μg/100 mg dry weight	202 ± 3	203 ± 4	212 ± 2	206 ± 2
DNA-phosphorus:				
Total (μg)	476 ±46	303 ±22**	311 ± 9	309 ±24
μg/100 mg dry weight	210 ± 8	256 ± 8**	210 ± 2	243 ± 5***
Total protein nitrogen (mg)	22·0 ± 2·0	10·5 ± 0·8***	14·6 ± 0·8	11·5 ± 0·8*

$$\text{† Percentage growth rate} = \frac{(\text{final live weight} - \text{initial live weight})}{\text{initial live weight}} \times 100$$

* Significant at $P = 0{\cdot}05$. ** Significant at $P = 0{\cdot}01$. ***Significant at $P = 0{\cdot}001$.

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Proteolytic enzymes and the clotting of milk in the stomach of the young pig. By R. Braude, A. M. Dollar, K. G. Mitchell and J. W. G. Porter, *National Institute for Research in Dairying, Shinfield, Reading*, and D. M. Walker, *Royal Veterinary College, Royal College Street, London, N.W.*1

Mean values for the proteolytic enzymic activity of stomach tissue, stomach contents, small-intestine contents and pancreas of pigs of different ages killed 2 h after suckling are shown in the table. The activity at pH's 1·6 and 3·5 was measured with haemoglobin and at pH 8·4 with casein as substrates.

Pigs		Stomach tissue m-equiv. tyrosine /g tissue/h*		Stomach contents m-equiv. tyrosine /pig/h*		Intestinal contents m-equiv. tyrosine /pig/h*		Intestinal contents g casein /pig/h†	Pancreas g casein/g tissue/h†
Mean age (days)	No.	pH 1·6	pH 3·5	pH 1·6	pH 3·5	pH 1·6	pH 3·5	pH 8·4	pH 8·4
4	7	0·05	0·10	0·08	0·11	0·45	0·11	0·67	1·8
13	10	0·08	0·08	0·15	0·08	2·5	0·34	2·4	1·3
22	8	0·29	0·42	0·08	0	1·4	0·55	2·5	2·1
29	8	0·52	0·45	0·14	0·08	2·6	0·88	6·4	2·3
36	6	1·2	0·92	0·08	0·18	0·86	0·75	6·8	4·7

* Released. † Digested

It is apparent that the proteolytic activity per gram of stomach tissue increases slowly during the first 2 weeks and then more rapidly. However, the proteolytic activity of the stomach contents remains relatively constant. One possible explanation is that the increased activity secreted into the stomach of the older pigs passes with the chyme into the duodenum, thus causing the observed increase in proteolytic activity at pH's 1·6 and 3·5 in the intestinal contents. The proteolytic activity at pH 8·4 in the pancreas and in the contents of the small intestine increases with age.

The weight of dry stomach contents increased from birth to about 2 weeks and decreased during the 3rd and 4th weeks. This finding was confirmed in a further experiment in which at weekly intervals the stomach contents were examined of pairs of piglets killed 15 min. after suckling. At 1, 2, 3 and 4 weeks the mean weights of dry contents were 12, 56, 24 and 15 g, respectively. The high values were associated with the presence of a hard cheese-like clot. Milk in the stomachs of very young pigs does not form a hard clot; such a clot is formed by pigs 2–3 weeks old but by 30 days of age the clot is again soft and disperse. The clotting of milk and the retention of the clot in the stomach may be a necessary mechanism to allow efficient digestion by the small amounts of proteolytic enzymes secreted during the first 2–3 weeks of the pig's life.

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