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ABSTRACTS OF COMMUNICATIONS

The Seventy-fifth Meeting of The Nutrition Society was held at the Linnean Society's Rooms, Burlington House, Piccadilly, London, W. 1, on Thursday, 15 May 1952, at 2 p.m., when the following papers were read:

Electrolyte–Water Relationships as a Means of Determining Changes in Body Water Content. By K. L. BLAXTER and J. A. F. ROOK, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Storage of body fat can be determined from the equation: fat stored = body-weight gain – (protein + ash + water) stored. The lack of accurate methods for the estimation of water storage has limited this method to qualitative studies (Keys, Brožek, Henschel, Mickelsen & Taylor, 1950; McCance & Widdowson, 1951). A method for the accurate determination of water is now described. It is based on the assumption that the osmotic pressure of body fluids is constant and that sodium and potassium contribute a fixed portion of this osmotic pressure. Determinations of the sodium, potassium and water contents of muscle, fat, liver, erythrocytes, posterior chamber fluid and pericardial fluid of Ayrshire cattle, aged from 1 week to 4 years, gave the empirical relationship:

$$\text{H}_2\text{O (g)} = 0.2922 \text{ Na (mg)} + 0.1471 \text{ K (mg)}.$$

The standard error of estimate was $\pm 2.0\%$. No significant differences were observed between animals or between the above tissues. Brain and serum water were over-estimated by 13% by this equation. The water of rumen, small intestine and caecum contents, of whole foetuses and of amniotic fluid was estimated accurately by the equation, but that of abomasal contents and of allantoic fluid was underestimated by 50%. Because of the discrepancies, water storage calculated from the above equation would, generally, be in error by about 0.5%, but during pregnancy an error of 12% could arise.

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The Indirect Estimation of the Water Balance of Animals as a Basis for the Determination of Energy Exchanges. By K. L. BLAXTER and J. A. F. ROOK, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Prediction of water retention from body storages of sodium and potassium was studied in metabolism experiments with calves. Five experiments were conducted in a closed-circuit respiration chamber in which continuous determinations of oxygen consump-

tion, carbon-dioxide and water-vapour production, mineral, nitrogen and energy balances were made throughout periods of 8 days. Water retention, estimated by the classical balance technique, was compared with that estimated from simultaneous determinations of sodium and potassium retention (Blaxter & Rook, 1952). The results agreed within $\pm 3\%$. Fat retention determined from carbon and nitrogen balances was compared with that estimated from gains of body-weight, protein, water and ash. In these short-term experiments the indirect estimation of fat retention was complicated by inaccuracies encountered in weighing the animal, but in later experiments, in which a more satisfactory weighing technique was employed, the error was substantially reduced and was shown to decrease linearly with increasing length of experimental period. Provided that sufficiently long experimental periods are employed, these results suggest that metabolic balances of minerals and nitrogen may be used to estimate energy retention, and, if combined with determinations of the heats of combustion of foods and excreta, the total energy exchange.

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Vitamin C Reserves in Patients in a Mental Hospital. By Z. A. LEITNER,
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For the purpose of this study 173 patients (103 male and 70 female) were selected from a larger series. Saturation tests were carried out according to the procedure of Harris & Abbasy (1937) for varying periods up to 7 consecutive days using 2:6-dichlorophenolindophenol as a reagent. In the present series only 8% were saturated on the 2nd, 25% on the 3rd day; 67% were saturated on the 4th day or after, 40% on the 5th day or after, 16% on the 6th day or after and 7% on the 7th day or after. In confirmation of the findings of Harris & Olliver (1943) there was a definite seasonal difference. Thus, the majority of patients in the spring series became saturated on the 5th and 6th day, in the autumn series on the other hand between the 3rd and 5th day. The length of stay in hospital had a pronounced influence; about 70% of the patients in hospital for less than 2 years were saturated on the 2nd and 3rd day, whereas among patients admitted for 10 years or longer only 18% became saturated during this time. Whilst only about 8.5% of the patients admitted for less than 2 years failed to reach saturation until the 5th day, with patients in hospital for 10 years or more the corresponding figure was 58%.

On the basis of this preliminary study it is indicated that a large proportion of patients who had been for long periods in a mental hospital had a low vitamin C status.

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Histochemistry of Succinic Dehydrogenase. By A. H. MALATY and G. H.BOURNE, *Department of Histology, London Hospital Medical College, London, E. 1*

Succinic dehydrogenase can be demonstrated histochemically by the method of Seligman & Rutenburg (1951). It depends upon the fact that if fresh frozen sections of tissues are incubated in succinate plus a suitable buffer and ditetrazolium chloride, the ditetrazolium acts as a hydrogen acceptor and becomes reduced to, and precipitated as, an insoluble blue diformazan pigment.

We have investigated this reaction in a number of tissues and have found that it was difficult to obtain any result with the tissues of animals which had been killed by anaesthesia. Good results were obtained from those of animals which had been killed by a blow on the head. Best results were obtained with kidney and skeletal and cardiac muscle, but liver gave poor results. Nervous tissue was intermediate.

The reaction was granular and the shape, size and distribution of the granules was identical with those of mitochondria.

In cardiac and skeletal muscle the reaction was localized in the sarcomes, which are derived from mitochondria. These results further support the conception of the mitochondrial localization of the enzymes responsible for aerobic metabolism of cells.

REFERENCE

Seligman, A. M. & Rutenburg, A. M. (1951). *Science*, **113**, 317.

The Home Preservation of Runner Beans Using Iodized and Other Salts.By H. R. HINTON and DOROTHY F. HOLLINGSWORTH, *Scientific Adviser's Division, Ministry of Food, Great Westminster House, Horseferry Road, London, S.W. 1*

A preliminary experiment in 1950 showed that French beans preserved by the dry-salting method and stored for 2 months with free-running salt, iodized or non-iodized, were tougher, according to taste-panel judgement, and greener in colour than beans preserved with kitchen salt. The pH of the liquor from the beans preserved with the free-running salts was much higher than that from beans preserved with kitchen salt. Iodized salt produced the highest pH.

The experiment was repeated in 1950-1 with runner beans which were stored for 9 months. The earlier results were confirmed but this time the iodized free-running salt had a more marked effect on toughness and colour than the non-iodized salt. It also produced a liquor of slightly higher pH than non-iodized salt in either experiment and the iodized salt in the first.

The findings of the second experiment were confirmed in a more extensive experiment conducted in 1951-2, which showed in addition that the amount of pectin extractable from the beans varied inversely with the pH. This observation suggests that the calcium and magnesium salts present in free-running salts, and causing the increased pH, react on the pectin in the beans to form insoluble calcium and magnesium pectates. It is suggested that these are deposited in the middle lamella which is then not dissolved away on cooking.

There was complete destruction of ascorbic acid in all samples.

The Effect of Thiouracil and Thyroxine on the Composition of Milk. By R. CHANDA, MARY L. MCNAUGHT and E. C. OWEN, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Thiouracil caused a decrease in the lactose and an increase of the chloride in milk. Thyroxine had the opposite effect. An increase of chloride accompanied a decrease of lactose with advancing lactation in untreated cows. In both treated and untreated animals there was thus the expected close negative correlation between the lactose and the chloride so that the freezing point of the milk of all cows was constant, irrespective of treatment. Neither thiouracil nor thyroxine affected the creatine or creatinine in milk in spite of the marked increase, caused by thyroxine in urinary creatine (Owen, 1948). Creatine phosphate was not present in milk. Calcium in the milk was unaffected by either drug thus contrasting with phosphorus which was increased by thyroxine (Owen, 1948; Chanda & Owen, 1951) and decreased by thiouracil (Chanda & Owen, 1951). The expected effect of thyroxine in increasing milk yield was observed and fat content also increased. Thiouracil depressed the yield. Neither drug affected the sodium, potassium or magnesium in the milk.

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- Chanda, R. & Owen, E. C. (1951). *Biochem. J.* **50**, 100.
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Vitamin A, Carotenoids and Tocopherol Levels in the Blood of Two Different Classes of Patient. By Z. A. LEITNER, *Claybury Hospital, Woodford Bridge, Essex, and 52 Welbeck Street, London, W. 1*, and T. MOORE and I. M. SHARMAN, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Vitamin A was estimated by $SbCl_3$, carotenoids by yellowness and tocopherol by $FeCl_3$ in blood plasma collected for a year in London from healthy private patients, and in Claybury from Mental Hospital inmates. Table 1 shows that the mental

Table 1. *Mean values for March 1951–February 1952*

	Private patients			Mental patients		
	No. of subjects	Range	Mean	No. of subjects	Range	Mean
Vitamin A (i.u./100 ml.)	219	51–297	148	159	12–364	118
Carotenoids ($\mu g/100$ ml.)	220	15–371	133	179	18–137	53
Tocopherol (mg/100 ml.)	64	0.60–2.91	1.31	175	0.56–3.25	1.15

patients averaged slightly lower than the private patients for vitamin A and tocopherol, and much lower for carotenoids. There were only minor seasonal variations. Male private patients, of mean age 46 years, had vitamin A higher and carotenoids lower than in females, aged 44 years. These sex differences confirm general experience. In the

mental patients, however, both vitamin A and carotenoids were slightly higher in women, aged 69 years, than in men, 49 years. Analysis of the data by age failed to explain the unexpected vitamin A superiority in female mental patients. Possibly sex only influences vitamin A levels at liberal carotenoid intakes. The carotenoid levels now reported for private patients exceed previous British values, but are lower than in U.S.A. Vitamin A and tocopherol are in the same ranges as in U.S.A. Statistical calculations are proceeding.

A Suggested Modification of the 'Rat Growth' Method of Protein Evaluation. By K. J. CARPENTER and G. PORTER, *Rowett Research Institute, Bucksburn, Aberdeenshire*

As the growing rat requires some protein for maintenance, the 'protein-efficiency ratios' of diets (body-weight gain \div total protein eaten) can be disturbed by differences in palatability with *ad lib.* feeding (see, for example, Mitchell, 1944; Graves, 1945). The variance of individuals is also high (Harte, Travers & Sarich, 1947). Paired-feeding techniques are unsuitable as the heavier animal has the greater maintenance requirement.

In an attempt to overcome these objections we have used: (1) A fixed range of growth (65–80 g), in contrast to other methods having a fixed period of growth. (2) A preliminary period of adaptation to the experimental diets (from weaning, at 45–52 g, up to 65 g, with *ad lib.* feeding to 60 g body-weight). (3) A scale of daily feeding according to body-weight (7.0 g/day for rats of 60 g, +0.3 g/day for every 2 g over this weight)—the highest possible for diets of medium palatability.

The variance between rats has been such that a 10% difference between two rations could be detected ($P > 0.95$) with eight rats/treatment. As in all methods for the determination of 'protein-efficiency ratios' the values are relative to one another rather than absolute (cf. Hegsted & Worcester, 1947; Bureau of Biological Research, 1951), and will vary with the level of protein used in the diets (Barnes, Maack, Knights & Burr, 1945). They may also, because of variations in composition of the body-weight gained, differ in their ranking from 'net protein values' based on nitrogen retention (Mitchell, 1944).

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Mitchell, H. H. (1944). *Industr. Engng Chem.* (Anal. ed.), **16**, 696.

The Supplementary Protein Value of a By-product from Grass Processing.

By K. J. CARPENTER, J. DUCKWORTH and GABRIELLE M. ELLINGER, *Rowett Research Institute, Bucksburn, Aberdeenshire*

The moisture content of fresh grass intended for drying was reduced to 60–70% by an expelling operation. A by-product, Graminex, was obtained by heat coagulation from the expressed liquor (Dawson, 1951). It contained negligible crude fibre and 33–55% crude protein, with a high lysine and methionine content important in supplementary proteins (cf. Carpenter, 1951).

	Analytical data (g/16g N)		Value in mixed rations					
	Lysine	Methionine	Chicks		Rats			
			'Gross protein value'	Protein-efficiency ratio	Protein-efficiency ratio	True digestibility*	Biological value for growth*	Net protein value*
White fish meal	6.1	2.9	90	1.88	3.81	90	68	61
Groundnut meal	3.4	0.9	50	1.68	2.83	86	55	47
'Graminex'	5.2	1.8	56	1.22	2.35	80	63	50
Grass meal	4.9	1.6	55	†	†	†	†	†

* Mitchell, 1944. † Not available.

The 'gross protein value' (Heiman, Carver & Cook, 1939) of Graminex for chicks (3% supplementary protein) was superior to that of groundnut meal. With practical 19% protein rations (11% supplementary protein) it was inferior. The Graminex ration was then laxative, and protein and energy were less completely digested. With rats, using the same rations diluted to give 9.5% total protein, the protein-efficiency ratios followed the same trend. However, the 'net protein value' of the Graminex ration exceeded that with groundnut meal. In no case did Graminex approach fish meal in value.

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