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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Fifty-first Meeting of the Nutrition Society (One Hundred and Thirty-seventh of the Scottish Group) was held in the Biochemistry Department, University of Glasgow, on Wednesday, 1 *October* 1980 *when the following papers were read:*

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Effects of oestrone on apparent vitamin B_6 status in peri- and post-menopausal women. By D. A. BENDER[1], W. F. COULSON[1], L. PAPADAKI[2] and M. PUGH[3], [1]*Courtauld Institute of Biochemistry and* [2]*Bland Sutton Institute of Pathology, The Middlesex Hospital Medical School, London W1P 7PN, and* [3]*The Hospital for Women, London W1V 6VB*

Administration of oestrogenic steroids as contraceptives leads to changes in tryptophan metabolism that are indicative of vitamin B_6 deficiency or depletion (Rose, 1978). In order to test whether oestrogens given as hormone replacement in the menopause, and endogenous oestrone secretion, also affect apparent vitamin B_6 status, tryptophan metabolism and other indices of vitamin B_6 status have been measured in thirty peri- and post-menopausal women who were attending the menopause clinic at the Hospital for Women, London.

When the patients were receiving no oestrogen therapy, the urinary excretion of 'total oestrone' (largely oestrone glucuronide, measured by radio-immuno-assay) was positively correlated with the excretion of kynurenine and negatively correlated with the plasma concentration of pyridoxal phosphate (which was determined chemically). There was no correlation between the excretion of oestrone and that of xanthurenic and kynurenic acids, or the degree of activation of plasma aspartate aminotransferase by pyridoxal phosphate added in vitro. The excretion of 4-pyridoxic acid, the principal metabolite of vitamin B_6, was positively correlated with oestrone excretion, and negatively correlated with the plasma concentration of pyridoxal phosphate.

Fourteen of these women subsequently received piperazine oestrone sulphate (Harmogen, Abbot Laboratories Ltd.), 3 mg/d, to control their menopausal symptoms. After 6 months' treatment, indices of tryptophan metabolism and vitamin B_6 status were again assessed, and in each case the results were compared with those obtained from the same woman before the start of treatment. Results were evaluated by the paired *t*-test. There was a significant decrease in the plasma concentration of pyridoxal phosphate, and an increase in the excretion of kynurenine. There was no significant change in the excretion of xanthurenic and kynurenic acids or in the activation of plasma aspartate aminotransferase by added pyridoxal phosphate.

These results are not compatible with the depletion of vitamin B_6 by either endogenous or administered oestrogen, but suggest a more specific effect on the metabolism of tryptophan, probably inhibition of kynureninase (L-kynurenine hydrolase, *EC* 3.7.1.3), either by competition between the steroid and pyridoxal phosphate for the apo-enzyme, as suggested by Rose (1978) or by some other mechanism (Wynick & Bender, 1981).

Rose, D. P. (1978). Vitamins & Hormones (1978) **36**, 53.
Wynick, D. & Bender, D. A. (1981). *Proc. Nutr. Soc.* **40**, 21A.

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The effect of oestrone sulphate on the activity of kynureninase in vitro.

By D. WYNICK and D. A. BENDER, *Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 7PN*

Women taking preparations of oestrogenic steroids show changes in tryptophan metabolism that are compatible with inhibition of the enzyme kynureninase (L-kynurenine hydrolase, *EC* 3.7.1.3) (Bender *et al.* 1981). Such changes have been reported frequently, and have generally been interpreted as indicative of vitamin B_6 deficiency induced by the steroids (Rose & Adams, 1972). However, other indices of vitamin B_6 status, such as plasma pyridoxal phosphate concentration, urinary excretion of 4-pyridoxic acid and the metabolism of a test dose of methionine, are generally unaffected (Leklem *et al.* 1975). It has been suggested that the steroids or their metabolites compete with pyridoxal phosphate for the cofactor binding site of apo-kynureninase, and thus reduce its activity (Mason & Gullekson, 1960).

A partial purification of kynureninase from rat liver has been affected by selective heat denaturation and ammonium sulphate fractionation. The cofactor was removed by brief incubation with phenylhydrazine, followed by extensive dialysis. This led to almost total loss of catalytic activity, which was restored by the addition of pyridoxal phosphate. Kynureninase activity was determined by the method of Bender & Smith (1976).

Oestrone sulphate was used as a conveniently available oestrogen conjugate. In the presence of a saturating concentration of pyridoxal phosphate, oestrone sulphate inhibited kynureninase, showing primarily competitive kinetics with respect to kynurenine, and a K_i of 82 ± 6 μM. With respect to pyridoxal phosphate, the inhibition was primarily uncompetitive. Preliminary studies indicated that oestrone glucuronide inhibited kynureninase in the same way as did the sulphate. Unconjugated oestrone was not inhibitory.

Addition of a saturating concentration of pyridoxal phosphate to rat liver homogenate led to a sevenfold increase in the activity of kynureninase, suggesting the presence of a relatively large amount of apo-enzyme under normal conditions. This would explain the normalization of tryptophan metabolism in women taking oestrogens by supplementary vitamin B_6 (Rose & Adams, 1972).

These results do not support the hypothesis that oestrogen metabolites displace pyridoxal phosphate from enzymes, but suggest that the abnormal tryptophan metabolism which has been observed in women taking oestrogenic steroids may be the result of direct inhibition of kynureninase by a metabolite of the steroid rather than the result of drug-induced sub-clinical vitamin B_6 deficiency.

Bender, D. A., Coulson, W. F., Papadaki, L. & Pugh, M. (1981). *Proc. Nutr. Soc.* **40,** 20A.
Bender, D. A. & Smith, W. R. D. (1978). *Biochem. Soc. Transact.* **6,** 120.
Leklem, J. E., Brown, R. R., Rose, D. P. & Linkswiler, H. (1975). *Am. J. clin. Nutr.* **28,** 535.
Mason, M. & Gullekson, E. H. (1960). *J. biol. Chem.* **235,** 1312.
Rose, D. P. & Adams, D. W. (1972). *J. clin. Pathol.* **25,** 252.

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The voluntary food intake of sheep when silage juice is infused into the rumen. By E. JILL SMITH and J. L. CLAPPERTON, *The Hannah Research Institute, Ayr KA6 5HL*

The voluntary food intake of forage material conserved in silage is generally less than that of the same material conserved as hay or dried grass and this effect has been ascribed to a physical limitation of appetite (Thomas *et al.* 1976). Clancy *et al.* (1977) showed that rapid infusion of silage juice (2·25 l) into the rumen of sheep that were given alfalfa hay, reduced rumen motility and stopped the animals eating during the first part of the day, although much of the deficit was made up later.

Two Finn × Dorset wether sheep fitted with a permanent rumen cannula were given long hay and two similar sheep were given frozen grass *ad lib.* Water was infused (2 l) on days 1 and 2 of each week and silage juice (2 l) infused on days 3 and 4. The quantity used was about equal to that contained in half the expected voluntary intake of silage and the juice was obtained by mincing the silage and passing the minced material through rollers. In three successive weeks, the juice was infused over a period of 0·75, 1·5 and 3·5 h respectively.

(Values are means with their standard errors; no. of determinations in parentheses)

		Water infused* (12)		Silage juice infused					
				0·75 h (4)		1·5 h (4)		3·5 h (4)	
Diet	Period (h)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Hay	4	295	24	63	35	40	22	108	35
	8	467	44	158	56	106	47	204	31
	24	1000	33	1057	75	649	107	724	59
Frozen grass	4	366	20	187	57	311	58	267	25
	8	642	40	370	70	587	56	478	38
	24	1000	12	756	54	947	32	883	21

*Mean of all infusion times.

The cumulative food intakes of the animals (expressed relative to the mean food intake when water was infused) are shown in the Table. The infusion of silage juice reduced the food intake during the first 4 h especially in the sheep fed hay but, after this, the rate of eating was almost normal and the total daily food intake was only reduced by 19 and 14% of the normal on the hay and frozen grass respectively.

Clancy, M., Wangsness, P. J. & Baumgardt, B. R. (1977). *J. Dairy Sci.* **60,** 580.
Thomas, P. C., Kelly, N. C. & Wait, M. K. (1976). *J. Br. Grassld. Soc.* **31,** 19.

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Incubation of hay with rumen micro-organisms; two distinct phases of digestion. By USHA R. MEHRA, *IVRI, Izatnagar* 243122, *India,* J. W. CZERKAWSKI and GRACE BRECKENRIDGE, *The Hannah Research Institute, Ayr KA6 5HL*

The Dacron bag technique (Mehrez & Ørskov, 1977) has been used to estimate the digestion of feeds. Our own results and those reported by others (e.g. Smith & Clapperton, 1981) suggest that there may be two distinct phases in the disappearance of dry matter (DM) during incubation of hay with rumen micro-organisms. The object of the present experiments was to investigate the time-course of digestion of hay and to apply some of the concepts on compartmentation developed from Rusitec work (Czerkawski & Breckenridge, 1979).

The incubations were made in closed plastic jars (250 ml), with facility for the collection of gas. Chopped hay (5 g) was placed in nylon gauze bags as used in Rusitec, suspended in 150 ml of microbial suspension, prepared by mixing strained rumen contents (compartment 1) and micro-organisms that had been washed from solid digesta (compartment 2) and incubated, with shaking, in a water-bath at 37° for 1·5 to 48 h.

The disappearance of DM with time of incubation could be described accurately by two similar hyperbolic relations, one terminating and the other starting after 10 h of incubation (phases (a) and (b)) respectively. The disappearance of non-fibrous DM was largely complete during phase (a) and the disappearance of fibre (cellulose and hemicelluloses) was confined entirely to phase (b). The outputs of methane and volatile fatty acids (VFA) per unit loss of hay DM were abnormally low during phase (a) and some 40% higher than the accepted values during phase (b). The measurements of the release of carbohydrate and soluble protein and the distribution of microbial matter in the three compartments, made it possible to calculate the amounts of DM actually digested. This resulted in considerable simplification, the amounts of DM digested being a linear function of time up to 31 h of incubation (r 0·998). During both phases of reaction the production of methane and VFA was directly proportional to the amount of DM digested and during phase (b) the values of mol methane:kg DM digested and VFA:kg DM digested (1·8 and 7·9 respectively) were similar to the values obtained in sheep given hay and during incubation of hay in Rusitec. During phase (a) of the reaction the amounts of methane and VFA produced/kg DM digested were lower and resembled those associated with concentrate digestion.

The results are consistent with a simple model in which during phase (a) there is a rapid microbial colonization and digestion of substrates in compartment 2 and in which the microbial colonization of compartment 3 (solid matrix) and the digestion of fibre may commence only when phase (a) is complete.

Czerkawski, J. W. & Breckenridge, G. (1979). *Br. J. Nutr.* **42,** 229.
Mehrez, A. Z. & Ørskov, E. R. (1977). *J. agric. Sci., Camb.* **88,** 645.
Smith, J. & Clapperton, J. L. (1981). *Proc. Nutr. Soc.* **40,** 22A.

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The effect of xylose on xylanase (hemicellulase) activity in the cell free liquor prepared from an artificial rumen (Rusitec). By R. E. BRICE and I. M. MORRISON, *The Hannah Research Institute, Ayr KA6 5HL*

Little is known concerning the mechanisms regulating the production and activity of carbohydrases in mixed populations of rumen micro-organisms. The following experiment was designed to examine the effect of xylose (the ultimate product of xylanase digestion of hemicellulosic cell wall material) on xylanase activity in the cell free liquor prepared from Rusitec (Czerkawski & Breckenridge, 1977).

Three vessels were infused continuously with soluble food, which contained minerals, casein, amino acids and carbohydrates, namely soluble starch, cellobiose and glucose each at 0·05% (w/v). Each vessel also contained three bags of 3 g chopped hay. One bag was changed every day for a fresh bag of chopped hay so that the time of residence, in the vessel, for each bag was 3 d. The chopped hay had been washed with warm water to remove soluble sugars from the plant material and then freeze-dried before being weighed out in the nylon bags. The cell free liquor, assayed for xylanase activity, was prepared as follows. The bags of digested chopped hay removed every day from each vessel were washed with artificial saliva, pH 6·9 (McDougal, 1948). The washings from each bag were pooled separately, 10 ml samples taken, and centrifuged at 2000 *g* for 45 min. The resulting clear supernatant was assayed for xylanase activity (Morrison, 1976). After 9 d the glucose in the soluble food infusion was replaced in vessel 2 by a mixture of xylose and glucose each at 0·025% (w/v) (the 'low xylose' food), and in vessel 3 by xylose at 0·05% (w/v) (the 'high xylose' food).

By day 16 xylanase activity in vessel 3 ('high xylose' food) had been reduced by approximately 17%. However, in vessel 2 ('low xylose' food) there was a 25% increase in xylanase activity.

These results suggest that whilst high levels of xylose may reduce xylanase activity, at lower concentrations xylose increases xylanase activity in the cell free liquor. These changes in xylanase activity were not, however, accompanied by corresponding changes in the digestion of the chopped hay. The digestion of the chopped hay in vessels 1, 2 and 3 was 62·5%±2·1, 61·4%±1·8 and 60·8%±2·0 respectively, during this period. This observation suggests that xylanase activity is not a limiting factor in the digestion of cell wall material.

Czerkawski, J. W. & Breckenridge, G. (1977). *Br. J. Nutr.* **38,** 371.
McDougal, E. I. (1948). *Biochem. J.* **43,** 99.
Morrison, I. M. (1976). *Carbohyd. Res.* **47,** 129.

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Milk and heavy metal absorption. By J. QUARTERMAN and E. MORRISON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The absorption and retention of lead, iron, cadmium and manganese are known to be greater in rats or humans given only milk compared with subjects given a solid diet (Kello & Kostial, 1977; Saarinen & Simes, 1979). The reason is not known but there is evidence that it is due to a possible deficiency of Fe, induced by the whole-milk diet a property of a liquid diet and it is not *per se.*

This effect of a whole-milk diet, however, must be distinguished from the effect of milk given along with a solid diet. In this paper evidence is presented to show that milk had a much smaller or an inhibitory effect on absorption when accompanied by solid food.

Groups of six to ten rats weighing approximately 95 g were given a solid semi-purified diet and water, whole milk only, skim milk only, the solid diet with milk instead of water, or the solid diet and skim milk, to appetite for 2 d. On the third day each rat was given about 5 μCi ^{203}Pb or ^{59}Fe in 0·2 ml saline (9 g sodium chloride/l) by stomach tube and then given the diets they had been receiving previously for a further 2 d. They were then killed and activity measured in tissues and gut-free carcasses.

Effect of diet on retention (% of dose in gut-free carcass) of ^{59}Fe and ^{203}Pb

		Diet				
Metal		Solid	Milk	Skim milk	Solid + milk	Solid + skim milk
^{203}Pb	Mean	4·9	10·6***	7·5	3·3	2·2*
	SE	1·0	0·7	2·3	0·8	0·4
^{59}Fe	Mean	15·2	29·0***	29·3***	21·8***	18·0
	SE	1·2	1·7	1·8	0·7	1·7

*$P<0·05$, ***$P<0·001$ (with respect to solid diet).

The Table shows that the carcass retention of both Pb and Fe was increased when milk or skim milk only was given. Milk admixed with solid diet enhanced Fe retention only; skim milk plus solids failed to promote retention of either Pb or Fe.

Kello, D. & Kostial, K. (1977). *Toxicol. Appl. Pharm.* **40**, 277.
Saarinen, V. M. & Simes, M. A. (1979). *Pediat. Res.* **13**, 143.

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Relation between blood glutathione peroxidase activity and the response to injected selenium in cattle. By M. PHILLIPPO, J. PRICE and J. R. ARTHUR (Introduced by I. BREMNER), *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Calves on nineteen farms were injected with either 6 mg selenium or 2 ml control solution within 5 d of birth. Whole-blood glutathione peroxidase (GSHPx) activities were measured by the method of Paglia & Valentine (1967) in samples taken from control and Se treated calves 78–184 d after treatment.

The mean GSHPx activities in control animals on different farms varied from 0·76–22·64 units/ml whole blood. Statistical analysis showed that within the period 78–184 d GSHPx activity in treated animals did not depend on the interval between Se injection and sampling. Se treated animals had significantly higher whole-blood GSHPx activities than control animals on fourteen of nineteen farms.

The difference in blood GSHPx activity between control and Se treated animals was notably smaller on farms where the control GSHPx was less than 2·5 units/ml blood (=0·025 μg Se/ml blood). The relationship between control (x) and treated (y) GSHPx activity was best described by the equation;

$$y=3\cdot75\ x^{0\cdot631},$$ standard error of exponent $\pm0\cdot049$, RSD$\simeq$20%.

The observation that differences in GSHPx activity attributable to Se injection may be related to the Se status of comparable untreated animals suggests that when GSHPx activity is particularly low (i.e. 2·5 units/ml whole blood) demand for repletion of tissue reserves may take precedence over synthesis of the enzyme in erythrocytes. This possibility warrants further investigation.

Paglia, D. E. & Valentine, W. N. (1967). *J. Lab. clin. Med.* **70**, 158.

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Assessment of vitamin status during total parenteral nutrition in surgical patients. By P. STROMBERG, A. SHENKIN, R. CAMPBELL, R. J. SPOONER and A. J. W. SIM*, *Departments of Biochemistry and Surgery**, *Royal Infirmary, Glasgow*

Vitamin status was assessed in patients with post-operative complications at the start and finish of thirty-nine courses of total parenteral nutrition (TPN; mean duration 16·7 d, range 4–51 d). The daily regimen was standard and consisted of 14 g nitrogen, 12·6 MJ (3000 kcal; 2000 as dextrose, 1000 as 20% intralipid), minerals and trace elements. The daily vitamin supplementation was one ampoule of Solivito and Vitlipid (Kabi Vitrum).

The mean plasma level of vitamin A increased from (mean ± 1 SD) 1·43±1·00 μmol/l to 1·95±1·22 μmol/l ($P<0·05$; Wilcoxon Signed Rank Test, n 36). Twenty out of twenty-nine remained low (<2·0 μmol/l) at the end of TPN. In three patients vitamin A concentration fell to subnormal levels (normal range 2·0–5·0 μmol/l) by the end of treatment. In all three patients treatment was complicated by infection or further surgery or both. Two patients developed elevated plasma levels of between 5·5–6·5 μmol/l by the end of therapy.

The mean plasma concentration of vitamin E increased from 20·1±11·3 μmol/l to 28·8±11·1 μmol/l during treatment ($P<0·02$, n 28). All low plasma levels (<14 μmol/l) were increased to normal values and no low levels developed during TPN. However, four patients developed elevated levels of between 42–58 μmol/l.

Status of vitamin B_1 was assessed by activation of erythrocyte transketolase. One patient was deficient (27%) at the start (range <25%) but was corrected by 5 d TPN. Two patients became deficient during therapy with activations of 28 and 34% being observed after 17 and 7 d respectively.

Serum folate levels increased during therapy from 1·67±1·02 to 2·30±1·44 μg/l ($P<0·05$, n 15). Of twelve patients who started with low serum levels (<2·2 μg/l) two patients also had low red cell folates of 81 and 77 μg/l (normal range >100 μg/l). Serum folate was not corrected in seven patients by the end of TPN.

No abnormalities of vitamins B_2, B_6 and B_{12} were recorded at any time although vitamin B_6 status improved (within the normal range) during therapy ($P<0·025$).

Evidence of vitamin depletion is common at the start of TPN. For folic acid and vitamin A the standard supplements used did not consistently correct all cases of deficiency and, indeed, some patients developed low levels during treatment. Increased levels of vitamin supplements may, therefore, be necessary and vitamin status should be regularly assessed.

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The relationship between habitual energy intake and body-weight on polar stations. By I. T. CAMPBELL, *Royal Liverpool Hospital, Liverpool L7 8XP*

A number of energy intake studies have been conducted on static scientific stations in the Arctic and Antarctic (Masterton *et al.* 1957; Milan & Rodahl, 1961; Easty, 1967; Garshenin, 1971; Ohkubo, 1972). The results of these surveys are summarized in the Table.

Mean energy intake, mean body-weight and mean body-weight changes over 1 year at polar scientific stations

	Energy intake (MJ)	Body-wt (kg)	Wt change (kg)
Masterton *et al.* (1957)	16·3	79·3	—
Milan & Rodahl (1961)	18·7	81·5	+4·9
Easty (1967)	15·0	74·6	+2·7
Garshenin (1971)	16·1	77·8	+3·1
Ohkubo (1972)	11·5	64·05	+3·0
Acheson (1974)	13·6	70·3	−1·0
Campbell (1975)	13·8	69	+1·6

An energy balance survey was carried out at a scientific station (Halley Bay, Antarctica 75°31′S 26°42′W) by two observers working independently (Acheson, 1974; Campbell, 1975). The mean daily energy intake of eleven subjects measured monthly, for a week at a time, over a period of 6 to 12 months, was 13·7 MJ. Mean body-weight was 69·7 kg. This intake is lower than most of those previously described but so is the body-weight. No satisfactory relationship has been established between body-weight and habitual energy intake in individuals. On a polar station populated by healthy young men the experimental population is more homogeneous with respect to age, diet, activity and lack of pathology than under more conventional circumstances. The correlation coefficient of energy intake and body-weight of Acheson and Campbell's subjects was 0·715 which was significant ($P<0·001$). The correlation coefficient for Easty's subjects was 0·626 ($P<0·001$) (n 25). The correlation coefficient of all individual intake and body-weights available in the published work outlined above was 0·697 ($P<0·001$) (n 40).

It is concluded that body-weight is a major factor in the determination of habitual energy intake on polar scientific stations. The habitual energy intake of a reference 70 kg man on a polar station, derived from the data described above, should be in the region of 13·5 MJ/d.

Acheson, K. J. (1974). PhD Thesis. University of London.
Campbell, I. T. (1975). MD Thesis. University of London.
Easty, D. L. (1967). *Br. J. Nutr.* **21**, 7.
Garshenin, V. F. (1971). *Voprosy Pitaniya* **5**, 32.
Masterton, J. P., Lewis, H. E. & Widdowson, E. M. (1957). *Br. J. Nutr.* **11**, 346.
Milan, F. A. & Rodahl, K. (1961). *J. Nutr.* **75**, 152.
Ohkubo. (1972). *Bull. Toky Med. Dent. Univ.* **19**, 245.

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