The Two Hundred and Ninety-fourth Scientific Meeting of the Nutrition Society was held in the Edward Lewis Lecture Theatre, Middlesex Hospital Medical School, London W1P 7PN, on Friday, 21 May 1976, at 10.00 hours, when the following papers were read:

The effect of dietary sucrose on the metabolism of pentobarbitone. By A. H. NASH and A. E. BENDER, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Previous work (Bender, Damji & Ismail, 1973) has shown that rats fed on a sucrose diet have shorter 'sleeping times' (duration of anaesthesia) following intraperitoneal injection of pentobarbitone than starch-fed animals. Dickerson, Basu & Parke (1971), however, measured the in vitro activity of some drug-metabolizing enzymes and found a decreased specific activity of biphenyl 4-hydroxylase and cytochrome *P*-450 in the livers of rats fed on sucrose.

Two hypotheses to explain this effect were examined: (a) that it was due to a change in distribution of the drug brought about by a change in carcass composition; (b) that the drug was metabolized at a faster rate.

Fourteen pairs of litter-mate rats (WAGS) were assigned randomly to two groups and given diets with either 600 g sucrose or 600 g starch/kg for 4 weeks. Thereafter 'sleeping times' were determined, in the morning at weekly intervals. The mean differences in sleeping time between litter-mate pairs of rats were:

Weeks		Difference (min)													Median
Fed (A)	4, 5	33	3	46	25	−11	50	2	1	62	18	−119	6	3	15	10
Fasted 24 h (B)	6, 8, 10	4	0	33	−6	−17	43	−19	6	34	2	4	9	−18	20	4
Fed (C)	7, 9, 11	34	20	22	19	1	45	−5	10	41	9	22	17	5	34	21

P (one-tailed): A, <0·02; B, <0·12; C, <0·001; A *v.* B, <0·03; A *v.* C, <0·32; B *v.* C, <0·002.

At weeks 6, 8 and 10 food was removed 12 h prior to injection; effectively a 24 h fast. At all other times the two diets were given *ad lib.*

Fasting increased sleeping time of starch-fed animals by 24% and sucrose-fed animals by 35% ($P<0.001$) and abolished the differences between the two groups. The rapidity of these changes tends to discredit hypothesis (a).

Injections were discontinued at week 11. At week 14 the animals were again anaesthetized and killed in pairs at 20 min intervals from 40 to 300 min: plasma pentobarbitone was determined (De Bruijn, 1974). In eleven of the fourteen pairs the plasma concentrations were lower in the sucrose-fed animals. When these points are plotted as semi-logarithmic decay curves the steeper slope obtained for sucrose supports hypothesis (b), namely, that the rate of metabolism of pentobarbitone is enhanced.

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A defective response to cold in the obese (obob) mouse and the obese Zucker (fafa) rat. By P. TRAYHURN, P. L. THURLBY and W. P. T. JAMES, *Dunn Nutrition Unit, University of Cambridge and Medical Research Council, Milton Road, Cambridge CB4 1XJ*

Davis & Mayer (1954) reported that genetically obese (obob) mice have an impaired response to cold; at 3° the mice died of hypothermia within, on average, 3 h. With recent interest in thermogenesis as a possible major factor in energy regulation (Miller, 1975) we have looked further at the response to cold stress of both the obob mouse and the Zucker (fafa) rat.

Obese mice, aged 3–9 months, placed in a room at 4°, had a rapid fall in core temperature and died after 5–6 h when their temperature had fallen to 12°. The lean mice, however, after 24 h at 4° still had core temperatures within the normal range. A similar study on adult (4–12 months) Zucker rats showed that the obese individuals also became hypothermic on removal to the cold, and the average survival time of five animals was 28 h. Death again occurred when the body temperature had fallen to 12°. No significant decrease was observed in the temperature of lean rats kept at 4° for up to 72 h.

The oxygen consumption of lean and obese mice, aged 3–9 months, was measured both at 22° (the temperature at which they were kept in the animal house) and on their removal to 4°. On transfer to the lower temperature the oxygen consumption of the lean mice increased threefold within a few minutes and this increase was sustained for the 6 h for which measurements were made. Although the oxygen consumption of the obese mice also increased in the cold, the increment was only one-half of the increment found for their lean litter-mates. These results are at variance with those of Davis & Mayer (1954), who found no increase in the oxygen consumption of obese mice in the cold.

Our results show that despite the increased insulation from an excess of body fat the genetically obese animals are very susceptible to cold stress. The impaired metabolic response to cold demonstrates that abnormalities in vasoconstriction are not responsible for the hypothermia, and suggests that the metabolic basis for the obesity could relate to impaired thermogenesis. Impaired thermogenesis appears not to be a secondary feature of genetic obesity since we have been able to detect it in obob mice prior to the phenotypic expression of obesity; from this a test has been derived for the early identification of obob individuals.

P.L.T. acknowledges the receipt of a Research Studentship from the MRC.

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Estimation of heat loss from human subjects at four experimental temperatures, using a direct calorimeter and heat-flow meters.

By W. H. CLOSE, M. J. DAUNCEY and D. L. INGRAM, *ARC Institute of Animal Physiology and MRC Dunn Calorimetry Group, Babraham, Cambridge CB2 4AT*

Heat-flow meters have been used to monitor the rate of heat loss (HL) from man and animals under laboratory and field conditions (Wever & Aschoff, 1957; Ingram, Heal & Legge, 1975). The meters record the potential difference proportional to the temperature difference across them and thence the heat flow. Using radiotelemetry, they provide the opportunity to record the HL of freely moving human subjects. The aim of the present study was to compare an estimate of HL using heat-flow meters with measurements of total HL in a direct calorimeter (Close & Mount, 1975).

HL from human subjects (three males and three females aged 22–55 years) was determined while each sat in a calorimeter wearing a cotton boiler-suit, minimal underwear and footwear. Simultaneous estimates of HL from the trunk were obtained using four Hatfield heat-flow meters (Hatfield & Wilkins, 1950) contained in a belt and fastened around the waist. HL was recorded on four separate days for each subject at environmental temperatures of 15, 20, 25 and 30° (±0·5°). Measurements were made for 2 h in the morning and for 2 h in the afternoon at each temperature. Results (kJ/m^2 per h; mean values with their standard errors) were:

Environmental temperature	Meter HL		Calorimetric HL: Total		Calorimetric HL: Sensible		Calorimetric HL: Evaporative		Ratio, meter HL: total HL
Males	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
15°	223	20	227	6	198	5	29	1	0·98
20°	180	10	202	10	158	7	44	3	0·89
25°	133	14	180	15	108	7	72	9	0·74
30°	79	4	187	12	61	4	126	8	0·42
Females									
15°	227	20	187	3	162	3	25	2	1·21
20°	173	15	169	6	130	6	39	3	1·02
25°	119	5	140	3	86	4	54	4	0·85
30°	83	3	119	5	47	4	72	4	0·70

The partition of the total heat loss into its sensible and evaporative components is expressed per unit of surface area (Du Bois & Du Bois, 1916). Total heat loss was significantly higher ($P<0·05$) for the male subjects at each environmental temperature. This was a consequence of their significantly higher ($P<0·05$) sensible heat loss; only at 30° was the evaporative heat loss of the male subjects higher ($P<0·05$) than that of the females. The heat loss recorded by the heat-flow meters was similar for both males and females at each environmental temperature.

The results suggest that heat-flow meters on the trunk may provide a reasonable estimate of total heat loss only where sensible heat loss accounts for a major proportion of the total.

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Fat mobilization during exercise following dietary and exercise-induced changes in muscle glycogen. By C. WILLIAMS, R. J. MAUGHAN, G. R. KELMAN, DORIS M. CAMPBELL and DIANNE HEPBURN, *Department of Physiology, University of Aberdeen, Aberdeen AB9 1AS*

The glycogen concentration in human skeletal muscle can be increased by a combination of exercise and carbohydrate-rich diet (Bergström & Hultman, 1966). This procedure has been shown to increase the capacity for prolonged heavy exercise (Saltin & Hermansen, 1967).

In a previous study (Kelman, Maughan & Williams, 1975) we confirmed that carbohydrate metabolism during exercise is significantly increased after glycogen loading procedures. The present study was undertaken to determine the influence of the glycogen loading procedures on fat mobilization during prolonged heavy exercise.

Four healthy male volunteers underwent the following exercise and dietary procedures in order to increase their muscle glycogen concentration. On the first day the fasting subjects exercised to exhaustion on a bicycle ergometer at 75% of their maximum oxygen uptake. During the following 3 d the subjects ate a diet low in carbohydrate, after which they again exercised to exhaustion. This was followed by 3 d on a high-carbohydrate diet and again by exercise to

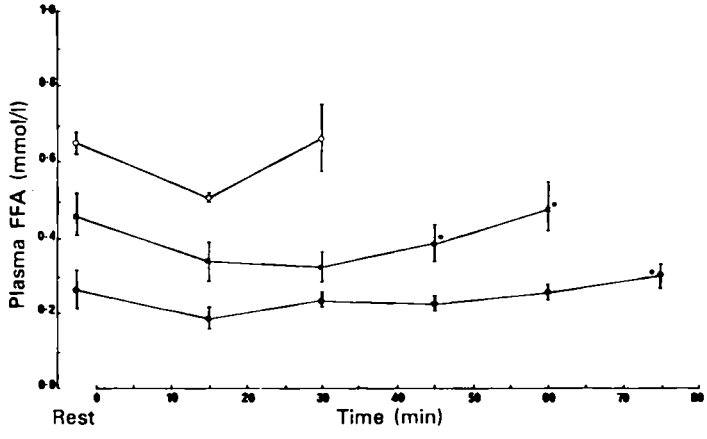

Fig. 1. Plasma free fatty acid (FFA) concentrations in men during exercise following normal (×) and low- (○) and high-carbohydrate (●) diets. Values are means for four subjects (*value for three subjects) with their standard errors indicated by vertical bars.

exhaustion. Blood was sampled at rest and during the exercise periods for the determination of blood lactate and glucose, and plasma free fatty acids (FFA), glycerol and total proteins. Expired air samples were collected during exercise for the determination of respiratory exchange ratios (R). In an additional study the effectiveness of the above procedures in modifying the muscle glycogen concentration was confirmed using a percutaneous needle biopsy technique (Bergström, 1962).

After 30 min of exercise following the high-carbohydrate diet, the R value was significantly higher ($P<0.05$) than after the normal and low-carbohydrate diets, and remained significantly higher ($P<0.02$) throughout the exercise period. The resting plasma FFA concentration following the high-carbohydrate diet was significantly ($P<0.05$) lower than the value obtained after the low-carbohydrate diet. These differences persisted during exercise (Fig. 1); however there was no significant difference between the plasma glycerol concentrations during exercise following the three dietary conditions.

The results of this study suggest that fatty acid re-esterification may be enhanced, rather than lipolysis impaired, as a result of the dietary manoeuvres necessary to increase the concentration of muscle glycogen. This decrease in fatty acid availability, rather than the increase in muscle glycogen, may be responsible for the increase in carbohydrate metabolism during exercise.

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Time of glucose syrup ingestion to alleviate initial exercise hypoglycaemia. By J. D. BROOKE, *Department of Human Kinetics, University of Guelph, Guelph, Ontario N1G 2W1, Canada,* K. LLEWELYN, *Human Performance Laboratory, University of Salford, Salford 5, Lancs,* and L. F. GREEN, *Beechams Products, Applied Research Department, Leatherhead, Surrey*

In fasting human subjects, the onset of exercise results in a transient decrease in the blood glucose concentration (Dringoli, Ravaioli, Orsucci & Ciampolini, 1969), sufficient for hypoglycaemic symptoms to occur (Keele & Neil, 1971). On the other hand the ingestion of glucose syrup prior to exercise results in an increase in the blood glucose concentration during physical work and improved performance (Brooke, Davies & Green, 1972). In an attempt to eliminate the reported initial exercise hypoglycaemia, an experiment was conducted with four adult male subjects to assess the most appropriate time for the pre-work ingestion of glucose syrup.

After a 30 min rest period, subjects exercised using a leg ergometer at 150 W for 20 min, a work schedule which induced a heart rate of 140–150 beats/min. Following habituation and an initial trial with no feeding, a drink of 300 ml glucose syrup (100 g dextrose monohydrate (1·43 MJ)) was given at 0, 10, 20, or 30 min before exercise in a design balanced for treatments to subjects over the trials. Trials per subject were 1 week apart. Capillary blood was sampled every 5 min during rest and exercise (Green, 1972) and analysed by the glucose oxidase method of Gawehn, Wielinger & Werner (1970). The results are shown in Fig. 1.

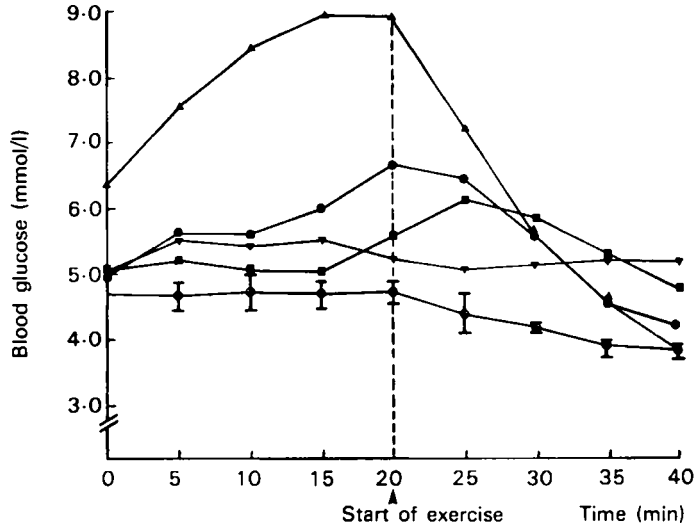

Fig. 1. Mean blood glucose values in four adult male subjects during exercise after ingestion of glucose syrup (G): ○, G not given; ▼, G given at start of exercise; ■, G given 10 min before exercise; ●, G given 20 min before exercise; ▲, G given 30 min before exercise. Vertical bars indicate standard deviations of mean values for four subjects.

With no feeding, the transient hypoglycaemia reported previously was observed. It was alleviated fully by ingestion of glucose syrup at the start of exercise. As the time of ingestion before exercise was lengthened, there was an increasing and earlier increase in the peak blood glucose concentration, followed as exercise progressed by a subsequent decrease towards the lower values observed in the subjects not given glucose syrup. It appeared that intelligent use of glucose syrup demands evaluation of the behavioural needs and appropriate timing of ingestion.

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Role of glucocorticoid in the enzyme overshoot response to starvation-refeeding. By CAROLYN D. BERDANIER, R. L. WURDEMAN and R. B. TOBIN, *Departments of Biochemistry and Medicine, University of Nebraska College of Medicine, Omaha, Nebraska* 68105, *USA*

Recent experimental work has shown that when rats are starved for 48 h and then given a diet with 650 g glucose/kg, hepatic lipogenesis will be greatly increased during the 2nd day of refeeding (Tepperman & Tepperman, 1958). This increased lipogenic capacity is characterized not only by an increase in fat synthesis, but also by an 'overshoot' in the activities of glucose-6-phosphate dehydrogenase (G6PD) (*EC* 1.1.1.49) and malate dehydrogenase (decarboxylating) (*EC* 1.1.1.40). The overshoot has been shown to result from de novo enzyme synthesis (Szepesi & Freedland, 1969). Although it has been shown that the overshoot of G6PD is dependent on the presence of dietary glucose, little information is available on the triggering mechanism responsible for this overshoot.

Since starvation is a stress to which the animal responds by an increased release of glucocorticoids, we postulated that these hormones could trigger the enzyme overshoot. This hypothesis was tested in a study of the enzyme responses of adrenalectomized (ADX) and intact rats to starvation–refeeding. Six groups of ADX and intact rats were used for this study. Four groups of ADX and intact rats were starved for 48 h and then given a diet with 650 g glucose/kg for 48 h. The first group of ADX and intact rats were given glucocorticoid during the starvation period (0·75 mg cortisol/kg body-weight subcutaneously every 12 h). The second set of ADX and intact rats were given the hormone during the refeeding period only. The third set of animals were given the hormone during both periods. The fourth set of animals received a placebo injection. A fifth set of ADX and intact rats were killed after the starvation period, and the sixth set of rats were given the diet with 650 g glucose/kg *ad lib.* The animals were killed by decapitation and the livers quickly excised, chilled, weighed, and a portion used for the determination of G6PD and malate dehydrogenase activity. Starvation resulted in a decreased enzyme activity in both ADX and intact rats. After refeeding, the ADX rats had the same enzyme activity as the control rats, whereas the intact rats had the expected enzyme overshoot. When ADX rats were given the hormone replacement, they too had the enzyme overshoot. Since the overshoot did not occur in the absence of cortisol and was due to de novo enzyme synthesis (Szepesi & Freedland, 1969), these results clearly implicate glucocorticoid as a triggering agent for the increased enzyme synthesis. These findings suggest that the stress response, i.e. the glucocorticoid response, plays an important role in the induction of the hyperlipogenesis associated with starvation–refeeding.

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The effect of ethanol on resting metabolic rate. By KATHRYN ROSENBERG and J. V. G. A. DURNIN, *Institute of Physiology, University of Glasgow, Glasgow G12 8QQ*

There is considerable disagreement about the effect of alcohol on metabolic rate. Several authors, for example Atwater & Benedict (1902), Le Breton & Tremolières (1955), and others, found no increased metabolic rate after alcohol ingestion in human subjects, but animal studies such as those by Shrimpton (1972) and Perman (1962) have noted increases of 10–15%. Other workers have also described similar or larger increases in man (Nagamine, Tezuka, Yamakawa & Suzuki, 1961; Stock & Stuart, 1974).

The interactions of alcohol and food may be complex. Studies have been made on ten female subjects, ranging in age from 21 to 37 years. Each subject received one of three types of meals on different days. After an overnight fast and a 30 min rest upon arrival in the laboratory, two or three resting measurements of oxygen consumption were taken by collection and analysis of expired air in a Douglas bag. One of the three meals was then consumed within 10 min. These consisted of: (a) 2·5 MJ (600 kcal) in the form of sandwiches with 230 ml red wine, (b) identical sandwiches with the wine replaced by an isoenergetic fruit drink, and (c) 230 ml red wine consumed on its own. The composition of the sandwiches was chosen to be representative of a 'mixed diet' and contained 15% of its energy as protein, with the remaining energy made up equally from carbohydrate and fat. The alcohol dosage varied between 0·3 and 0·4 g/kg body-weight. After the meal, measurements of oxygen consumption were made at 15 min intervals for a total of 3 h.

Although there were large variations between individuals in the magnitude of the effect, all subjects showed an increase in oxygen consumption after the wine alone. The duration of the effect was shorter in most instances than the reponse to the food, varying from 1 to 3 h before the metabolic rate returned to the preprandial level. Both the increase after wine consumption and the shorter duration of the effect were significant ($P<0.01$).

No significant difference could be detected in the magnitude of the response to the meal with alcohol as compared to the meal without. However, there was a tendency for the increased metabolic rate to persist longer after the meal which included alcohol.

K.R. is the holder of an MRC studentship.

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The fermentation of polyalcohols by rumen microbes in vitro. By E. POUTIAINEN, M. TUORI and IRMA SIRVIÖ, *Department of Animal Husbandry, University of Helsinki, Helsinki, Finland*

The observation that the use of xylitol in diets decreases the dental caries incidence in man by inhibiting the growth of bacteria on teeth (Schein & Mäkinen, 1974) has aroused interest in the possible use of polyalcohols or alditols in animal feeding in Finland. Xylitol is produced commercially and is used as a sweetening substance. The mixture of polyalcohols is the by-product of hydrogenated birch-tree hydrolysate.

Investigations were made of the activity of rumen microbes fermenting polyalcohols in vitro and the products of fermentation. The incubation was made by using the mixture of polyalcohols (composition (g/kg dry matter): xylitol 200, arabinitol 270, mannitol 200, galactitol 85, sorbitol 100, rhamnitol 80, other monosaccharides 30 and fermentation products 35) as well as the pure polyalcohols as substrate. The in vitro method of Tilley & Terry (1963) was applied excluding the stage of HCl–pepsin digestion. The rumen liquid used as inoculum was taken from two fistulated cows. Both were fed on roughage–concentrate diet (70:30, w/w) except that 1 kg polyalcohol mixture was infused into the rumen of one of the cows (Taakka) twice/d during 2 months prior to investigations. The incubation times were 2, 4, 8, 12, 24 and 48 h and the series was repeated twice for each cow. Determination of total polyalcohol was made by periodic acid titration (Tegge & Berghaller, 1970) and volatile fatty acids were measured by gas–liquid chromatography.

After 8 h incubation over 80% of the polyalcohols remained unfermented. However, after 24 h, although less than 20% of the xylitol and arabinitol had been

Table 1. *Molar percentage of volatile fatty acids in rumen liquid taken from two cows before and after 24 h incubation with various polyalcohols*

Carbohydrate source	Cow	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Caproic acid
Before incubation	Taakka	70·6	14·3	13·5	1·6	—
	Taava	70·3	15·9	12·2	1·7	—
Xylitol	Taakka	39·2	26·4	26·7	5·8	2·0
	Taava	64·6	16·3	15·5	3·6	—
Arabinitol	Taakka	38·5	25·6	24·1	8·7	3·1
	Taava	63·2	17·0	15·4	4·5	—
Galactitol	Taakka	23·7	24·3	43·4	6·1	2·5
	Taava	62·8	17·8	15·7	3·7	—
Mannitol	Taakka	22·2	38·2	33·5	5·8	0·4
	Taava	40·6	34·3	21·2	3·9	—
Sorbitol	Taakka	22·6	39·5	29·1	7·4	1·3
	Taava	38·4	46·8	12·7	2·1	—
Mixture of polyalcohols	Taakka	25·5	37·5	28·1	7·2	1·7
	Taava	51·4	30·9	14·9	2·8	—
Sucrose	Taakka	47·3	39·5	11·2	2·1	—
	Taava	48·6	37·2	12·4	1·9	—

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fermented, about 60% of the galactitol and mixed polyalcohols and almost all of the mannitol and sorbitol had been fermented. None of the polyalcohols was fermented as fast as sucrose. When 1 kg mixed polyalcohols was infused into the rumen of cow Taakka daily, excretion in the faeces was no greater than for normal diets. Thus it appears that the polyalcohols not fermented in the rumen are nevertheless absorbed and metabolized by the animal.

The molar percentages of volatile fatty acids after 24 h incubation (Table 1) show marked differences among the polyalcohols and also indicated that adaptation had occurred in the cow Taakka receiving the polyalcohols in the diet for 1 month.

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Pyridoxine deficiency in severe liver disease. By D. LABADARIOS, J. E. ROSSOUW, M. DAVIS and R. WILLIAMS, *Liver Unit, King's College Hospital and Medical School, Denmark Hill, London SE5*

Low circulating vitamin levels in patients with liver disease have been attributed, in the past, to malnutrition, since most of the patients studied were alcoholic (Leevy & Smith, 1974). In thirty-two patients with decompensated cirrhosis we have observed in 29% a deficiency of thiamin, in 88% a deficiency of pyridoxal phosphate (PLP), the biologically active form of pyridoxine, and in 14% a deficiency of ascorbic acid. The incidence was the same in fifteen patients with non-alcoholic liver disease as in eighteen alcoholics.

When intravenous supplements of thiamin hydrochloride (100 mg twice/d), pyridoxine hydrochloride (50 mg twice/d) and ascorbic acid (500 mg twice/d) were given for 1 week, plasma levels of PLP increased to normal in only 33%, although deficiencies of the other two vitamins were corrected. However, administration of the active coenzyme pyridoxal-5-phosphate (Roche Products Ltd, Welwyn Garden City, Herts.) resulted in an increase in plasma PLP levels, although the response was variable and less than in controls. This may be indicative of enhanced degradation or elimination, or both, of PLP in patients with cirrhosis, although impaired conversion of pyridoxine to PLP cannot be excluded.

PLP deficiency may also be related to leakage of the vitamin from damaged hepatocytes, for significantly higher than normal plasma levels of PLP were found in patients shortly after admission to hospital with fulminant hepatic failure (patients $106 \pm 14 \cdot 0$ (SE) ng/ml, controls $12 \cdot 6 \pm 1 \cdot 1$ ng/ml). Values subsequently fell to subnormal levels.

Because of the importance of pyridoxine in the metabolism of amino acids, whose disordered homeostasis may be important in the pathogenesis of encephalopathy, supplements of this vitamin should be given but in the form of the active cofactor pyridoxal-5-phosphate rather than the more usually administered and commercially available hydrochloride.

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An explanation of the paradoxical activities of α-retinol. By S. R. WILLETTS, SUSAN E. HOUGHTON, G. JONES and G. A. J. PITT, *Department of Biochemistry, University of Liverpool, PO Box 147, Liverpool L69 3BX*

α-Retinol (an isomer of retinol containing an α-ionone instead of a β-ionone ring) is very effective in inducing the signs of hypervitaminosis A, but has little growth-promoting activity (Houghton, 1969; Pitt, 1969). Although other workers (Goodman, Smith, Hembry & Dingle, 1974) have confirmed that α-retinol given orally has little activity in promoting growth, it is effective in reversing the characteristic lesion of vitamin A deficiency, keratinizing metaplasia of mucous epithelia, in hamster tracheas in organ culture (Clamon, Sporn, Smith & Saffiotti, 1974).

α-[11–^{3}H]retinol was synthesized by reducing α-ionylideneacetaldehyde with NaB^3H_4 to α-ionylideneethanol, and reoxidizing this with manganese dioxide back to tritiated α-ionylideneacetaldehyde; condensation with ethyl senecioate gave α-retinoic acid, which was reduced to α-retinol.

α-[11–^{3}H]retinol (40 μg) was given orally to each of four rats deficient in retinol but maintained on retinoic acid. The rats were killed after 4, 8, 24 and 72 h; only small amounts (4·6→0·4 μg α-retinol/l) were found in the plasma predominantly in the form of α-retinyl esters. In contrast, rats given only 1 μg [11, 12–$^3H_2$]retinyl acetate tended to have higher plasma concentrations, almost wholly in the alcohol form.

The feeding of large doses of α-retinol (5 mg/rat, spread over 3 d) to rats maintained on retinoic acid raised the plasma concentration of α-retinol compounds to only 180 μg/l, compared with 370 μg retinol/l in rats given the same dose of retinol, even though ample stores (763 μg) of α-retinol compounds were in the liver.

Muhilal & Glover (1975) have found that α-retinol did not combine in vitro with retinol-binding protein (RBP), the plasma carrier for retinol.

The apparent paradox that α-retinol has poor growth-promoting activity, whereas it is very effective in inducing hypervitaminosis A and can correct vitamin A deficiency in organ culture, can be explained by its low affinity for RBP.

The implication is that growth tests to establish the structural requirements for vitamin A activity may be measuring the ability of molecules to bind to RBP rather than the structural specificity for the molecular mode of action of the vitamin in tissues.

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The significance of the 'free' folate content of foods is questioned. By J. D. MALIN (introduced by K. MARY CLEGG), *Department of Food Science and Nutrition, University of Strathclyde, 131 Albion Street, Glasgow G1 1SD*

The folate activity of Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*) was determined with *Lactobacillus casei* as follows: the samples were macerated in phosphate buffer (pH 6·0) containing ascorbic acid as antioxidant (1·5 g/l) and then autoclaved at 115° for 10 min to aid extraction and to inactivate any native folate conjugase. Analysis of this extract measured the 'free' folate; the total folate was determined after a portion of the same extract had been treated with chicken pancreas extract under controlled conditions.

Sephadex G15 (Pharmacia Ltd, Uppsala, Sweden) gel chromatography of extracts of sprouts and subsequent co-chromatography with ^{14}C-labelled pteroylhepta- and pteroyltriglutamates revealed that large folate molecules predominated in the extract of cooked sprouts. On the other hand, several bands ranging from large polyglutamates to triglutamates and smaller folates were detected after fractionation of fresh sprout extract.

Incubation of homogenates of fresh sprouts with the two labelled folates, and subsequent gel chromatography of the extracts and analysis of the column fractions by autoradiography, indicated that some hydrolysis of the peptide chain in the labelled folates had occurred. These observations explain the quantitative anomaly (Table 1) when losses of 'free' folate are found after cooking with no corresponding alteration in the folate content. Native conjugases in the raw sprout had been mobilized by the maceration procedure and had caused significant hydrolysis of the polyglutamate folates before inactivation of the enzymes during autoclaving could take place; the 'free' folate of cooked sprouts was not over-estimated because the conjugase activity (previously detected in animal and vegetable tissue, including cabbage, by Tamura, Buehring & Stokstad (1972)) was destroyed during the cooking of the whole sprouts prior to the preparation of the extract.

Table 1. *The 'free' and total folate activities of fresh and cooked Brussels sprouts (*Brassica oleracea *L. var.* gemmifera*) determined by* Lactobacillus casei *assay*

(Mean values and standard deviations for six extracts/sample)

Sample	Moisture (mg/g)	'Free' folate (μg/g)		Total folate (μg/g)	
		Wet wt	Dry wt	Wet wt	Dry wt
Fresh	889	1·50±0·06	13·46±0·54	1·77±0·22	15·89±1·97
Cooked	906	0·03±0·03	0·32±0·32	1·46±0·20	15·47±2·12

Therefore, over-estimation of the 'free' folate of uncooked food may occur, with the apparent loss of this activity on cooking; in the past, such artificial losses have undoubtedly been attributed incorrectly to the thermal instability of folates. Thus, the use of 'free' folate assays for many fresh food materials is of questionable value.

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Altered morphology and pathways of DNA synthesis in small intestinal epithelium in dietary folate deficiency. By A. M. TOMKINS, J. BADCOCK and W. P. T. JAMES, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT, and Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ*

The significance of mild folate deficiency insufficient to cause anaemia, as described in many cases of acute tropical sprue (Tomkins, James, Cole & Walters, 1974), is unknown. However, intestinal morphological changes include villous atrophy with megaloblastic appearances of nuclei. Similar changes, with lower levels of DNA (mg/mm jejunum), can also be produced in the thinner intestine of weanling rats born to mothers on a folate-deficient diet (modified from Kodicek & Carpenter, 1950). These young animals developed only mildly lower levels of folate in erythrocytes and serum when maintained on the diet, and none became anaemic.

Using an in vitro incubation system for intestinal rings in Krebs–Henseleit buffer, both 'de novo' (folate-dependent) and salvage pathways for DNA synthesis were found to operate in mucosal cells. In mucosa from normal animals the addition of deoxyuridine (UdR) in concentrations of 0·25 μM, 2·5 μM, 0·025 mM and 0·25 mM suppressed the uptake of ^{3}H-labelled thymidine (TdR) (5 μCi [^{3}H]TdR/2 ml incubation fluid) into DNA to 91, 75, 26 and 20% of control values respectively. In mucosa from folate-deficient animals UdR (0·25 mM) failed to suppress [^{3}H]TdR incorporation into DNA (mean value with standard error for six animals 96·7±5·9% of control) but the addition of folic acid (0·28 mM) to the incubation permitted UdR to suppress to levels achieved by normal animals (mean with SE for six animals 58·7±6·3% of control; value for normal animals (mean with SE for six animals) 50·4±4·6% of control).

These alterations in TdR uptake together with the high mucosal RNA:DNA ratio suggest that mild folate deficiency may interfere sufficiently with thymidylate synthesis to cause appreciable 'maturation arrest' in developing mucosal cells.

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Influence of Dieldrin on the production and characteristics of the egg and utilization of calcium and phosphorus in the quail (*Coturnix coturnix japonica*). By G. VARELA, M. ANDUJAR and M. P. NAVARRO, *Instituto Nutricion, Consejo Superior de Investigaciones Cientificas, Campus Facultad Veterinaria, Madrid-3, Spain*

In birds, the organochlorated pesticides have been associated with a decrease in egg production and fertility, rupture of the eggs, weakness of the shell etc., effects

which could be related to the altered utilization of calcium, as suggested by Peakall (1969) among others. Such alterations could influence the utilization of alimentary Ca and phosphorus; Varela, Torralba & Escrivá (1975), and Andujar (1976) have already described modifications in the utilization of other nutrients in the rat following ingestion of organochlorated pesticides.

We have performed the present study on adult quails (*Coturnix coturnix japonica*), males and laying females, fed for 48 d on a diet (Ca 32·4 and P 7·2 g/kg) to which was added 20 mg Dieldrin/kg. Control birds were fed on the same diet without Dieldrin. During the last 8 d we measured over-all Ca and P balance, mineralization of the femur, and the production and characteristics of the egg (Table 1).

Table 1. *Effect of Dieldrin (20 mg/kg diet) on calcium and phosphorus in the quail*

		Utilized*/intake ×100		Retained†/intake ×100		mg/Egg		mg/g Femur	
		Ca	P	Ca	P	Ca	P	Ca	P
Females	Control	49·5	24·5	15·4	14·3	252	16·6	212	106
	Dieldrin	59·5	26·7	27·1	17·2	234	15·7	227	112
Males	Control	16·4	−0·2	—	—	—	—	184	88
	Dieldrin	17·1	−0·3	—	—	—	—	188	90

*Intake−excreted.
†Intake−eliminated (excreta+egg).

The total number of eggs was not affected significantly, but the treated animals did show a significant decrease in egg production towards the end of the first month. The weight, size and resistance of the shell was not affected but the structure and Ca content was.

In the female, the elimination of Ca by way of the excreta and eggs was decreased. The food intake was not affected, and consequently a significant increase in Ca retention occurred, and this caused an increase in the Ca and P content of the femur.

In males, on the other hand, Ca and P depositions in the femur did not change. No significant differences in P utilization were detected.

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Anthropometry in a Sudanese village; changes after 7 years. By M. Y. SUKKAR, J. R. KEMM, A. MAKEEN and M. HABEEB, *Department of Physiology, Medical Faculty, University of Khartoum, Khartoum, Sudan*

It has been suggested that nutritional factors contribute to the differences in heights and weights between children in developed and underdeveloped countries, and also to the secular increase in height and weight in European communities. An increasing standard of living might therefore be expected to decrease the differences between children in developed and underdeveloped countries and to exagerate the secular trend in height and weight.

El Kalakla is situated 24 km south of Khartoum on the white Nile. An anthropometric survey of the children in this village was performed in 1968 by Sukkar, Johnson, Gadir & Yousif (1971). Since that survey the standard of living has increased, incomes are higher and clean water supply, electricity, a hard-surface road and improved health care facilities have been provided. There has been little migration into or out of the village so it offered a rare opportunity to measure the changes which had taken place. Accordingly a repeat anthropometric survey was performed at the end of 1975. In 1968 age had to be estimated in 80% of the schoolchildren but in 1975 60% had birth certificates and data from children without birth certificates were excluded.

Very little change had taken place between the two surveys. In 1968 the mean height and weight of the pre-school children (6 months–6 years) were below the 10th percentile of the British standard (Tanner, Whitehouse & Takaishi, 1966) while in the schoolchildren (6–13 years) the mean heights were between the 50th and 10th percentiles and the weights were about the 10th percentile. In 1975 the mean weights for children of each age group were still on the 10th percentile and the mean heights in children above the age of 30 months were about the 25th percentile. Inspection of the data showed a small increase in the heights and weights of the pre-school children and a small decrease in the heights and weights of the older schoolchildren.

These results suggest that the subtle nutritional changes associated with development have not produced a marked change in height or weight within the period of 7 years. They further suggest that genetic constitution or other factors such as environmental hygiene which have not changed may be the main contributors to the differences in height and weight between Caucasian and Sudanese children.

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Variations in serum chemistry in normal subjects without changes in diet. By D. A. BENDER, *Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR*

Six normal volunteers (four males and two females, aged between 24 and 32) gave blood samples at 2-week intervals over a period of 32 weeks. Blood was

collected between 09.00 and 10.00 hours, and the subjects had neither eaten nor taken any beverage other than water since the night before. As far as could be ascertained, none of the subjects was taking any medication during the period of the experiment, and all consumed their normal diet.

Samples of blood were submitted for routine biochemical profile investigation using the Vickers MC–300 automated system of the Middlesex Hospital Medical School; samples were analysed together with the normal hospital work-load. None of the results obtained was outside the normal ranges established on the same instrument over a period including this experiment. However, in all parameters investigated a considerable amount of fluctuation was observed from week to week. The system involves a great deal of internal standardization, and it is probable that most of the variation observed was due to in vivo fluctuations rather than analytical errors. Results for serum protein, albumin, urea, uric acid and cholesterol are shown in Table 1.

Table 1. *Fluctuations in serum chemistry in six normal subjects over 32 weeks*

(Values given are the lowest and highest obtained for each subject, expressed as a percentage of the mean value for that subject)

	Subject						Deviation from mean value (%)	
	A	B	C	D	E	F	Mean	Maximum
Protein	91–105	96–107	93–105	96–105	94–109	95–108	3·2	9·0
Albumin	89–109	87–108	86–111	86–116	89–111	86–115	5·5	15·5
Urea	77–124	92–116	76–126	76–129	72–117	65–137	11·7	36·7
Uric acid	85–124	83–120	74–111	81–117	77–130	76–153	10·9	52·7
Cholesterol	85–113	97–108	83–115	87–109	95–108	83–110	5·9	17·5

Many workers report statistically significant changes in cholesterol and other serum parameters following dietary modification, frequently based on only a few blood samples from each subject. In view of the fluctuations reported here, it is perhaps pertinent to ask whether such results, although statistically significant, are physiologically meaningful.

Alteration of the immune response in mice by dietary factors: effects of irradiating, autoclaving and essential fatty acid deficiency of rodent diets. By P. B. MEDAWAR, RUTH HUNT, C. J. MEADE and J. MERTIN, *MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ*

Treatment of normal rodent diets by autoclaving or gamma-irradiation causes acceleration of skin allograft rejection, decreased incidence of tumours by methylcholanthrene and a higher threshold for neonatally induced immune tolerance. Mixing of autoclaved with normal diet (50:50, w/w) did not fully normalize the immune response. A possible explanation of this immunopotentiation was thought

to be the generation of a stimulatory factor by irradiating or autoclaving the diet. One possible candidate for such a factor might be a vitamin. Retinoic acid as an oxidation product of vitamin A had been shown by other authors to potentiate immune responses (Nelken, Pick, Gabriel, Bitterman & Bass, 1965; Krishnan, Bhuyan, Talwar & Ramalingaswami, 1974; G. Floersheim, personal communication). A further possibility might be the physical destruction of dietary factors that normally reduce the intensity of the immune response.

Suppression of the cell-mediated immune response and activation of the reticuloendothelial system function have been observed in mice treated subcutaneously or orally with essential fatty acids (EFA) suggesting EFA participation in immunoregulatory mechanisms. This hypothesis was supported by our finding that, conversely, EFA-deficient diet fed to mice causes acceleration of skin allograft rejection and decrease in tumour incidence and growth.

Comparing the results of the different experiments it appeared to us that EFA deficiency might be one of the causes of the immunopotentiation caused by irradiated or autoclaved diet. EFA double bonds can be altered or destroyed by physical means. Heating unsaturated fatty acids destroys their double bonds. Irradiation of EFA using moderate dosages will change the position (Mead, 1952) of double bonds or may lead to their autoxidation (Eberhagen, Klempay & Zöllner, 1965) or to the break-up of the EFA molecule (Lück & Kohn, 1961). In order to examine this assumption we have autoclaved EFA-deficient and its appropriate control diet. Acceleration of allograft rejection similar to that seen as a result of the deficient diet was observed with autoclaved control diet, whereas autoclaving EFA-deficient diet had no additional effect. The subcutaneous injection of linoleic acid could abrogate the effect of EFA deficiency.

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Determination of methionine in the seeds of legumes. By A. DUNCAN, A. MCINTOSH and GABRIELLE M. ELLINGER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

For many classes of livestock methionine is the limiting amino acid in legume-seed proteins. The methionine content may differ considerably between varieties. A recently-developed gas–liquid chromatography (GLC) method for methionine (Ellinger & Duncan, 1976) was adapted to rapid screening of legume seeds for methionine. Methylthiocyanate released from proteins during reaction with cyanogen bromide (CNBr) is a measure of intact methionine. γ-Glutamyl-S-methylcysteine, a constituent of many legume varieties, interferes; it also yields methylthiocyanate with CNBr although, normally, free S-methylcysteine does not.

Free S-methylcysteine also interferes in methionine determinations that are based on colour development with sodium nitroprusside. Preliminary extraction eliminated this interference with minimal losses of methionine and without hindering the subsequent reaction with CNBr. Ground beans (60 mesh) were steeped overnight in diethyl ether-saturated water. The resulting suspension was thoroughly mixed with ethanol to give a 70:30 (v/v) ethanol–water solution before centrifugation and decantation of the supernatant. Two further extractions with 70:30 (v/v) ethanol–diethyl ether-saturated water were followed by two extractions with methanol. The diethyl ether contained 5 mg hydroquinone/kg to prevent peroxide formation. Some CNBr-reactive material was lost during extraction. γ-Glutamyl-S-methylcysteine was found to interact with components of the seed testa, possibly as a result of thioether–polyphenol interaction as discussed by Synge (1975). This loss was prevented when seed-coats were removed before extraction. Bound methionine was not implicated. Highly reproducible methionine values (SD between replicates 0·059) were obtained by GLC from the extracted solids. Comparable values for the unextracted beans obtained by ion-exchange chromatography (IEC) after performic acid oxidation were originally less reproducible and substantially higher. The cause of the discrepancy was traced to the IEC method. Evaluation of the exceptionally low methionine sulphone peak was distorted by neighbouring baseline irregularities that were not revealed on the computer-linked analyser and by the very disparate areas of adjacent aspartic acid and methionine sulphone peaks. After correction of these sources of error, good agreement between the two methods was obtained for several bean varieties. Values for the GLC and IEC methods respectively were (g methionine/kg whole bean): kidney bean (*Phaseolus vulgaris* L.) 2·6 and 2·7, cowpea (*Vigna unguiculata*) 4·0 and 3·9, pigeon pea (*Cajanus cajan*) 4·0 and 4·1, lima bean (*Phaseolus lunatus* L.) 1·8 and 2·0, field bean (*Vicia faba* L.) 2·3 and 2·4, green gram (*Vigna aureus* L.) 3·9 and 3·9. Black gram (*Vigna mungo*) with values of 2·5 and 5·1, the sole exception to good agreement, is under further examination.

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The effect of dietary carbohydrate on the utilization of protein by the growing pig. By M. F. FULLER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The effect of dietary carbohydrate on the utilization of protein, the protein-sparing effect, is an important mechanism by which the relative rates of protein and fat accretion may be adjusted in relation to diet. A previous experiment with rats (Fuller, Boyne, Atkinson & Smart, 1973) described the general relationship of nitrogen retention to intakes of protein and starch varied over a wide range, using mixtures of fish flour and gelatin to supply protein of good or poor quality. A similar experiment with good-quality protein has now been done with pigs.

Diets comprising twenty-nine combinations of fish flour (0–800 g/d) and maize starch (0–1200 g/d) were supplemented with vitamins and minerals. All diets contained lipid (30 g/kg) and wood fibre (30 g/kg). The total daily food allocation varied from 0·06 to 1·6 kg/animal. Two or three castrated male pigs, with a mean body-weight during the experiment of 33 kg, were allocated to each regimen. They were kept in metabolism cages for 18 d: urine and faeces were collected for the final 11 d.

The mean apparent digestibility of N was 0·96 and was not significantly influenced by starch intake. Urine N was reduced with increasing starch intake in all circumstances; the reduction in urine N on adding 1200 g starch ranged from a minimum of 2 g when no protein was given to a maximum of 18 g when protein in excess of 220 g/d was given. At all protein intakes there was a diminishing marginal response to starch, with some response persisting up to the highest attainable starch intake. When more than 220 g protein/d was given, the increase in N retention per g starch added to the diet fell from a maximum of 38 mg with the first increment to a minimum of 4 mg at a starch intake of 1200 g/d. Expressed in relation to metabolic body-weight, the results were comparable to those obtained with rats given similar diets.

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The oxidation in vivo of uniformly and carboxyl-^{14}C-labelled methionine by young pigs. By E. R. CHAVEZ and H. S. BAYLEY, *Department of Nutrition, University of Guelph, Guelph, Ontario N1G 2W1, Canada*

The influence of nutritional regimen on protein metabolism can be studied by measuring the rate of catabolism of individual amino acids. One way in which this can be accomplished is to measure the release of $^{14}CO_2$ after a tracer dose of ^{14}C-labelled amino acids (McFarlane & von Holt, 1969). However, the interpretation of the results of such studies can be difficult, because of differences in initial dilution of the tracer dose (Chavez & Bayley, 1976) and in the recycling of the amino acid-C (Reeds, 1974).

In the present study pigs of approximately 10 kg live weight received a single intravenous dose of either L-[U-^{14}C]- or L-[1-^{14}C]methionine. They were confined in respiration chambers and the $^{14}CO_2$ released was absorbed in organic solvents and determined by liquid scintillation counting.

Similar doses of each labelled species (5 nmol) were administered (Table 1) but because of the differences in specific activities, more activity was administered in the uniformly labelled than in the carboxyl-labelled dose; however, similar amounts of total activity were recovered in the first 1 h of the experiment from both forms of the tracer dose. The recovery of activity remained high for the first 3 h from the pigs which had received the uniformly labelled dose, whereas the

Table 1. *Administration of L-[U-^{14}C]- or L-[1-^{14}C]methionine and recovery of activity in $^{14}CO_2$ in 6 h from young pigs*

	L-[U-^{14}C]methionine	L-[1-^{14}C]methionine
	Administered	
Dose (nmol)	4·8	5·2
Specific activity (disintegrations/min × 10^{-3} per nmol)	539	138
Total activity (disintegrations/min × 10^{-3})	2590	720
	Recovered	
	Total activity	Total activity
	disintegrations/min × 10^{-3} / % dose	disintegrations/min × 10^{-3} / % dose
Time after infusion (h)		
1	38 / 1·4	33 / 4·6
2	46 / 1·8	24 / 3·3
3	41 / 1·6	16 / 2·2
4	29 / 1·1	10 / 1·4
5	23 / 0·9	6 / 0·8
6	20 / 0·8	6 / 0·8
Total	197 / 7·6	96 / 13·3

recovery fell throughout the 6 h for the pigs which had received the carboxyl-labelled dose. Examination of the semi-log plots of activity released *v.* time indicated a simple, one-pool system for the release of activity from the carboxyl-labelled methionine, but a more complex, multipool system for the release of activity from the uniformly labelled methionine.

Calculation showed that the average specific activities of the methionine molecules being oxidized were $18·5 \times 10^3$ and $41·0 \times 10^3$ disintegrations/min per nmol for the carboxyl- and uniformly labelled methionine molecules respectively, indicating that approximately half of the $^{14}CO_2$ from the uniformly labelled methionine was derived from the carboxyl-C, and thus a substantial fraction of the other four C atoms in the methionine molecule were being incorporated into other compounds and retained in the tissues.

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