

fed DCP contained 9.6% of the total P intake but that of lambs fed MAP contained only 0.5% of the P intake. When Mg intakes were increased as MIX and MAP were included in the diets, elevated amounts of Mg were found in both urine and faeces and more Mg was apparently retained.

It is concluded that magnesium ammonium phosphate is an efficient source of P and Mg for ruminants.

## REFERENCE

Hemingway, R. G. (1963). *Proc. Nutr. Soc.* **22**, xvi.

*The One Hundred and Eighty-ninth Meeting of The Nutrition Society was held at the Sir Edward Lewis Hall, The Middlesex Hospital Medical School, Mortimer Street, London, W1, on Friday, 26 May 1967, at 10.30 am, when the following papers were read :*

**The distribution of polyethylene glycol in gut contents.** By M. J. MANNERS and D. E. KIDDER, *School of Veterinary Science, University of Bristol*

To check the validity of experiments using polyethylene glycol (PEG) as a marker in studies of digestion in the piglet, we have examined the effect of adding solutions containing glucose and PEG to gut contents in vitro.

A solution containing, per 100 ml, 25 g glucose, 0.42 g PEG. (*M* 4000) and 3.4 g NaF as a preservative was prepared. Samples of contents were taken from the stomach and various sections of the small intestine of piglets and each was divided into two portions. To one portion, the glucose-PEG solution was added before centrifuging, the mixture was then centrifuged and the supernatant fluid taken. The second portion of the same gut contents was first centrifuged and the glucose-PEG solution then added to the supernatant. Glucose and PEG concentrations were determined on the supernatant fluids prepared in these two ways and dry-matter determinations were done on all the samples, making it possible to determine the ratio of water to glucose and PEG in each sample of supernatant fluid.

Where the glucose-PEG was added to the whole stomach contents, although the glucose:PEG ratio was much lowered (Table 1), the glucose was always distributed uniformly throughout the water of the sample. PEG distribution, however, was not uniform in the water in the whole stomach contents, where higher concentrations were found in the water of the supernatant than would have been present if it had dispersed evenly throughout all the water in the sample. A similar but

Table 1. *Glucose : PEG in supernatant as a percentage of glucose : PEG in original solution added to contents or supernatant from gut contents of piglets (means and standard errors from four experiments)*

	Stomach	Small intestine			
		Proximal quarter	2nd quarter	3rd quarter	Distal quarter
Glucose-PEG added to whole contents	85.8 ± 1.5	92.5 ± 1.6	94.5 ± 2.5	97.1 ± 1.7	97.5 ± 1.8
Glucose-PEG added to supernatant	94.3 ± 1.7	95.8 ± 1.4	95.8 ± 2.1	97.8 ± 1.9	98.8 ± 1.9

smaller effect was noted in the whole contents of the first quarter of the small intestine. This effect could be due to the failure of the high-molecular-weight PEG to diffuse into the water in the swollen casein particles of the stomach contents. This explanation is supported by finding that addition of the dry diet to glucose-PEG solutions, followed by removal of semi-solid matter by centrifuging, caused a rise in PEG concentration, but no change in glucose concentration.

**Further observations on the effects of low-manganese intake on shell formation in pullets.** By MARGARET LONGSTAFF and R. HILL, *Royal Veterinary College, University of London*

In an earlier communication (Mathers & Hill, 1965) it was reported that poor-shelled eggs were produced by pullets given a low-manganese diet from about 4 weeks before the start of egg laying. A subsequent experiment was carried out to determine whether the low-Mn diet had to be fed before the start of egg laying to produce poor shells, and if so, for how long. A low-Mn diet (6-7 µg/g; Hill, 1965) was introduced at point of lay (about 21 weeks old), at 19, 18 or 16 weeks of age and the resulting shells compared with those obtained from birds given a high-Mn diet (106-107 µg/g) throughout.

Birds given the low-Mn diet from 16 weeks of age produced poorer shells in the first weeks of production than those given a high-Mn diet. Shells improved with the age at which the low-Mn diet was introduced, and no effect on shells was produced when the low-Mn diet was fed from the point of lay.

In contrast to these results and those reported earlier (Mathers & Hill, 1965), the low-Mn diet of Lyons (1939) produced poor shells whenever it was given during egg production, and poor shell formation continued for as long as the diet was fed. A further experiment was carried out using a low-Mn diet similar to that used by Lyons (1939) together with a fish meal-maize diet also low in Mn (6-7 µg/g), and the same low- and high-Mn diets mentioned above. These diets were fed from 17 weeks of age.

The mean percentage of shell-less eggs in the first ten eggs laid by each bird was: 1 for the high-Mn diet, 23 for the low-Mn diet, 19 for fish meal-maize, and 17 for Lyons.

Ten birds of the twenty on each of the diets were chosen at random and the first six whole eggs at start of lay were examined for shell strength and thickness.

Further groups of six eggs from each bird were taken after 1 and 7 months of egg production.

	Start of lay		After 1 month		After 7 months	
	Deformn. ( $\mu\text{m}$ )	Wt/area ( $\text{mg}/\text{cm}^2$ )	Deformn. ( $\mu\text{m}$ )	Wt/area ( $\text{mg}/\text{cm}^2$ )	Deformn. ( $\mu\text{m}$ )	Wt/area ( $\text{mg}/\text{cm}^2$ )
High-Mn	22.7	72.9	21.1	77.5	23.2	75.4
Low-Mn	29.8	64.6	22.7	74.1	22.6	75.3
Fish meal	30.0	66.1	22.8	75.2	23.3	74.9
Lyons	34.3	62.5	27.8	68.3	31.4	66.1
Overall statistical significance of treatment differences	$P < 0.01$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.01$	$P < 0.01$

At the start of lay, eggs from birds on all three low-Mn diets were significantly poorer in shell strength and thickness than those on the Mn-supplemented diet. After 1 month of egg production, only shells from the Lyons diet were significantly poorer than those from the Mn-supplemented diet. Shells from the low-Mn and fish-meal diets had improved to such an extent that they were as good as those from the high-Mn diet. This continued to be the pattern even after 7 months of egg production.

#### REFERENCES

- Hill, R. (1965). *Br. J. Nutr.* **19**, 171.  
 Lyons, M. (1939). *Bull. Univ. Ark. agric. Exp. Stn* no. 374.  
 Mathers, J. W. & Hill, R. (1965). *Proc. Nutr. Soc.* **24**, xxx.

**The contribution of sweets and sugar to the diet of some 14-year-old Glasgow schoolchildren.** By J. V. G. A. DURNIN and N. G. NORGAN, *Institute of Physiology, University of Glasgow*

**Factors contributing to the weight loss during intestinal coccidiosis infections in the fowl.** By R. A. PRESTON-MAFHAM and A. H. SYKES, *Wye College (University of London), Ashford, Kent*

It has been shown (Reid & Pitois, 1965) that intestinal coccidiosis (*Eimeria acervulina* sp.) causes a reduction in feed intake and a loss in body-weight over the acute period of the infection. Further experiments have been performed to determine what portion of the total weight loss may be attributable to this reduction in feed intake.

A group of six 8-week-old cocks was infected with 10 million sporulated oocysts per bird and the feed intake and body-weights recorded over a 14-day period. A similar group of uninfected controls was given the same feed intake as the infected birds on a per kg body-weight basis, on the following day. The control birds lost significantly less weight ( $P = 0.01$ ) than the infected birds over the period of weight loss due to the disease. The mean weight loss, with its standard error, of the infected birds was  $42.5 \pm 9.11$  g and that of the controls was  $30.0 \pm 4.66$  g, therefore approximately 70% of the weight loss of the infected birds could be accounted for by a reduction in feed intake.

Since the parasite is found in the epithelial cells of the intestine, it seemed probable that another effect of the disease might be an impairment of absorption.

The absorption of L-histidine, D-glucose and fluid from the small intestine of normal and infected cocks was measured using both 'in vivo' and 'in vitro' methods.

The results (Table 1) indicate a marked reduction in absorption of the substances measured, during the infection ( $P < 0.001$  for each experiment).

Table 1. *Absorption rate expressed in mg/g dry weight of gut per 30 min (mean values with their standard errors for each group)*

Substance	Method	Absorption rate	
		Controls	Infected birds
L-histidine	In vivo	0.758 ± 0.039 (10)	0.528 ± 0.059 (19)
L-histidine	In vitro	0.990 ± 0.076 (5)	0.343 ± 0.056 (14)
L-histidine	In vitro*	0.746 ± 0.044 (9)	0.256 ± 0.097 (9)
D-glucose	In vivo	12.06 ± 2.11 (3)	5.46 ± 1.41 (12)
D-glucose	In vitro*	34.30 ± 1.65 (14)	12.43 ± 0.88 (26)
Fluid	In vitro	1900.00 ± 167.0 (14)	410.00 ± 68.8 (26)

Figures in parentheses are the numbers of measurements in each group

\*Concentration gradient present initially.

It is concluded that intestinal coccidiosis infections produce a weight loss, which can be attributed partly to a reduction in feed intake and partly to a decrease in the absorption of nutrients.

Grateful acknowledgement is made to May & Baker Ltd, for a Research Studentship to R.A.P.-M.

#### REFERENCE

Reid, W. M. & Pitois, M. (1965). *Avian Dis.* **9**, 343.

#### **The effect of protein intake on changes in serum protein levels associated with high-carbohydrate diets.** By B. L. COLES, *Department of Physiology, Guy's Hospital Medical School, London Bridge, London, SE1*

It has been shown that changes in the serum protein pattern can be induced in healthy men on a high-carbohydrate diet even when the protein intake is adequate (Coles & Macdonald, 1966). In this experiment the carbohydrate was supplied as raw starch or sucrose, 7 g/kg body-weight per day. The subjects were male medical students with weights ranging from 63 to 80 kg and three isocaloric diets were tested, 18% protein with starch, 18% protein with sucrose and 9% protein with sucrose. On all three diets the serum albumin fell significantly during a 16-day experimental period. On the 18% protein diets no significant changes were observed in any other protein fraction, but on the 9% protein diet the  $\gamma$ -globulin increased significantly.

#### REFERENCE

Coles, B. L. & Macdonald, I. (1966). *Clin. Sci.* **30**, 37.

**Nitrogen losses in sweat and their relation to protein requirements in tropical countries.** By ANN ASHWORTH, *Medical Research Council, Tropical Metabolism Research Unit, University of the West Indies, Jamaica*, and A. D. B. HARROWER, *University of Edinburgh Medical School, Edinburgh*

Recently it has been suggested that protein requirements must be higher in tropical than in temperate countries because of the loss of nitrogen in sweat. These proposals have often been based on work with unacclimatized subjects under conditions which may cause stress. There is evidence, however, that the concentration of N in sweat decreases with acclimatization, and that stress can have the effect of increasing the urinary N output, which may mask any compensatory decrease in urinary N when sweating. Furthermore, the N losses are frequently calculated from the composition of arm sweat, and this may not be representative of the N from the whole body.

Our study had two objectives: to find the extent of N losses in sweat in a group of acclimatized people undergoing fairly strenuous, but natural physical work in a hot climate; and to determine whether the increased loss of N in sweat was compensated by a decreased urinary N output, as would be expected on physiological grounds.

Six fully acclimatized volunteers were given a diet supplying 50 g protein daily. The experiment lasted 20 days, with alternating 5-day periods of minimal and heavy sweating. N balance was measured throughout. During 6½ h work approximately 3 l. of sweat were lost, containing on the average 0.49 g N, 64 m-equiv. Na and 22 m-equiv. K. Sweat from the whole body was collected.

The N concentration in sweat was 0.20 mg/g, which is lower than that found by most other workers. We suggest that acclimatization was responsible for this low figure.

There was a marked decrease in urinary Na excretion during sweating, which compensated fully for the loss of Na in sweat. Renal compensation for K loss was less efficient. Because the total N loss in sweat was small, it was not possible to establish with certainty whether it was compensated by a reduced renal excretion of N. However, after the initial period the subjects were in N balance in spite of the relatively low protein intake.

We conclude that there is no evidence to suggest that heavy sweating under natural conditions in a tropical climate causes any significant increase in protein requirements.

**Some long-term effects of dietary sucrose on the serum lipids of female baboons.** By T. M. COLTART, *Physiology Department, Guy's Hospital Medical School, London, SE1*

It was shown that when adult male baboons were given a 75% sucrose diet for 17 weeks, there was an increase in the concentration of triglycerides in the fasting serum (Coltart, 1967). Also, after 13 weeks on the high-sucrose diet, the animals developed a threefold increase in the specific activity of the serum triglycerides

following a  $^{14}\text{C}$  sucrose meal (Coltart, 1967).

In view of the evidence that the conversion of sucrose into serum triglycerides is different in young men from premenopausal women (Macdonald, 1965), it was decided to investigate the effects of a high-sucrose diet on female baboons.

Six healthy female baboons, weighing 9–12 kg, were given a diet containing 75% sucrose, 18% calcium caseinate, and 7% dried yeast. Salts and vitamins were added and the whole diet was dissolved in water. The amount given to each animal was adjusted to keep its weight constant. While on the diet each animal continued to have a regular oestrus cycle. Fasting serum glycerides were estimated at intervals throughout the 17 weeks on the diet.

To study the incorporation of dietary sucrose into serum triglyceride a 4 g/kg body-weight sucrose meal was given by stomach tube to each animal before the start of the diet and after 13 weeks on the diet. This sucrose was uniformly labelled with 25  $\mu\text{C}$   $^{14}\text{C}$ . Blood samples were taken up to 5 h after the meal, and the radioactivity in the triglyceride fraction estimated in a scintillator counter.

### Results

1. At no time throughout the 17-week dietary period did the mean fasting triglyceride level of the female baboons differ significantly from the mean pre-diet level. This was different from that found in the male baboons on the sucrose diet in which the mean fasting triglyceride level was significantly greater than the mean pre-diet level in eight out of the nine occasions that blood samples were taken while on the high-sucrose diet ( $P < 0.025$ ).

2. Whereas after 13 weeks on the high-sucrose diet the male baboons showed a 2.9-fold increase in the specific activity of the serum triglycerides following a  $^{14}\text{C}$  sucrose meal, the female baboons showed only a 1.7-fold increase after the same length of time on a similar diet.

These findings therefore would support the view that there is a difference between the sexes in the metabolic conversion of dietary sucrose into serum triglyceride.

I am grateful to Cadbury Brothers, Bournville, for a research grant.

### REFERENCES

- Coltart, T. (1967). *Proc. Nutr. Soc.* **26**, ii.  
Macdonald, I. (1965). *Am. J. clin. Nutr.* **16**, 458.

**Effects of dietary carbohydrate on some liver enzymes.** By JANET AITKEN, D. ROBINSON and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London, W8*

Rats fed sucrose instead of starch as the dietary carbohydrate are known to show many differences both in the levels of lipids in the blood and in the activities of several enzymes in the liver and other tissues (Nagdy, Miller, Qureshi & Yudkin, 1966; Freedland & Harper, 1958; Krebs & Eggleston, 1965; Konishi, 1966). More recently it has been shown that the pyruvate kinase in the liver of rats is

increased between three- and five-fold when the diets contain sucrose instead of starch (Yudkin & Krauss, 1967).

There are several differences between sugar and starch that may account for these effects. For example sucrose contains fructose. It may also be more readily absorbed; thus causing more rapid and larger fluctuations in blood sugar.

We have examined some of the hepatic enzymes in male rats fed on a number of purified diets containing 60% by weight of carbohydrate. The carbohydrate was either entirely maize starch, glucose, fructose, sucrose, or varying mixtures of these. The enzymes examined were ketohexokinase (EC 2.7.1.3), hexosediphosphatase (EC 3.1.3.11) and aldolase (EC 4.1.2.7).

Their activities were measured in liver homogenates from adult animals which had received the diets for 8 days, or 77–100 days. In general the activities of the enzymes were lowest in the liver of rats fed entirely starch or glucose. Compared with these, the activities were up to 35% higher in rats fed the other diets, the difference being roughly in proportion to the amount of dietary fructose (free or in sucrose). The animals fed for 8 days only showed more pronounced differences than those fed for longer periods.

#### REFERENCES

- Freedland, R. A. & Harper, A. E. (1958). *J. biol. Chem.* **230**, 833.  
Konishi, F. (1966). *J. Nutr.* **89**, 329.  
Krebs, H. & Eggleston, L. (1965). *Biochem. J.* **94**, 3C.  
Nagdy, S., Miller, D. S., Qureshi, R. U. & Yudkin, J. (1966). *Nature, Lond.* **209**, 81.  
Yudkin, J. & Krauss, R. (1967). *Nature, Lond.* (In the Press.)

#### **Effects of dietary carbohydrates on the serum lipids of the rat.** By P. A.

AKINYANJU and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London, W8*

The experiments reported were designed to throw light on the possible mechanism by which dietary sucrose produces different levels of blood lipids from those produced by dietary starch. We have examined the levels of cholesterol, phospholipids and triglycerides in male rats fed diets containing 60% carbohydrate as starch, sucrose, or glucose, as a combination of starch with sucrose, or as invert sugar. The diets were given *ad lib.* for 83 days from weaning. When the diets contained sucrose, or glucose, or invert sugar, the triglyceride levels in the blood were some 50–90% higher than when the diets contained starch. Mixtures of starch with sucrose gave levels that increased with an increase in the proportion of the sugar. Thus, unlike the increase in hepatic enzymes reported in the previous paper, a rise in serum triglycerides may be produced by sugars which do not contain fructose. Molecular size might therefore be an important factor in determining the hyperlipaemic effect of sucrose. This is also the explanation we favour for the detrimental effect of sucrose on the insulin reserve (Cohen & Yudkin, 1967, unpublished).

**Precipitation of magnesium in association with phosphate under the conditions obtaining in the calf ileum.** By R. H. SMITH and A. B. McALLAN, *National Institute for Research in Dairying, Shinfield, Reading*

There appears to be no experimental evidence to support the commonly held view that magnesium is sometimes rendered partly unavailable in the ruminant gut by precipitation as the ammonium phosphate. Phosphate-dependent precipitation of Mg in the ileal digesta of the ruminant calf has been demonstrated (Smith & McAllan, 1966) but the mechanism was unclear.

We have examined *in vitro*, using simple inorganic solutions, the influence on Mg precipitation of permuted variations in calcium (0–24 mM), phosphate (2–18 mM) and ammonium (0–25 mM) concentrations and pH (6–8). These ranges cover the properties of nearly all the ileal digesta samples which we have examined. All experiments were carried out at 27°. The solutions all contained 8 mM-Mg, were adjusted to the same ionic strength (0.25) and were incubated, with shaking, for the same length of time (2 h) before ultrafiltration.

Some salient findings were as follows:

1. With neither Ca nor NH<sub>4</sub> present there was no Mg precipitation, whatever the phosphate concentration or pH.
2. There was no Mg precipitation at pH 6 and usually little at pH 6.5 whatever the concentration of Ca, NH<sub>4</sub> or phosphate.
3. With 18 mM-phosphate and at pH 7.5. (a) With no Ca, precipitation of Mg increased progressively with increasing NH<sub>4</sub> concentration (to 74% at 25 mM-NH<sub>4</sub>). (b) With 12–24 mM-Ca, NH<sub>4</sub> concentration had little effect on the Mg precipitated. (c) With no NH<sub>4</sub>, Mg precipitation increased with increasing Ca concentration up to a maximum (43%) at about 12 mM-Ca. It decreased with further increase in Ca concentration. (d) With 25 mM-NH<sub>4</sub>, precipitation decreased progressively with increasing Ca concentration (from 74% to 32%).
4. For any one set of conditions otherwise favouring Mg precipitation the precipitation was approximately proportional to the phosphate concentration and increased progressively with pH above about 6.5.

It can be concluded that precipitation of Mg with phosphate would be appreciable only in the most distal part of the ileum where the pH is above about 6.5–7.0 and then only with fairly high phosphate concentrations. Given these conditions it appears that considerable differences in Mg availability could be produced by variation in either Ca or NH<sub>4</sub> concentration, but that variations in NH<sub>4</sub> concentration would not have much effect unless the Ca concentration was very low.

REFERENCE

Smith, R. H. & McAllan, A. B. (1966). *Br. J. Nutr.* **20**, 703.

**Clinical aspects of anorexia nervosa.** By A. H. CRISP (introduced by G. L. S. PAWAN), *Academic Unit of Psychiatry, Middlesex Hospital, London, W1*

The view that primary anorexia nervosa is a phobia for and consequent avoidance



of normal adolescent weight and that it is characterized by a selective starvation of carbohydrate has been proposed elsewhere (Crisp, 1965*a,b*, 1967). Such patients come from backgrounds with specific nutritional characteristics and have come more than others to associate their psychosexual pubertal changes with increasing weight. In response to various adolescent crises they have tended to mobilize weight loss as a means of resolving such difficulties. In some ways they can be regarded as having regressed back into biological childhood.

Various feeding patterns develop as the illness progresses. During the early stages there is almost always a period of several months and sometimes years of persistent avoidance of eating commonly identifiable carbohydrate. Subsequently some patients come to periodically surrender to their increasing impulse to eat carbohydrate. This may lead them, through a convalescent phase intermittent bulimia, to recovery. Alternatively, they may begin to vomit in relation to their bulimic episodes and thereby continue to avoid significant weight gain. Such behaviour may be secret and often denied.

A group of thirty-seven patients with this disorder have been found to have significantly higher serum cholesterol levels than thirty-seven 'normal' subjects matched individually with the patients for age and sex (Crisp, 1965*b*; Crisp, Blendis, Pawan & Lace, in preparation). Particularly high levels have been found to be most commonly associated with the pattern of intermittent bulimia.

#### REFERENCES

- Crisp, A. H. (1965*a*). *J. psychosom. Res.* **9**, 67.  
Crisp, A. H. (1965*b*). *Proc. R. Soc. Med.* **58**, 814.  
Crisp, A. H. (1967). *J. psychosom. Res.* **11**, no. 1. (In the Press.)

**Dietetic aspects of anorexia nervosa.** By ANNE E. LACE (introduced by G. L. S. PAWAN), *Dietetic Department, The Middlesex Hospital, London, W1*

The object of this study was to assess the practical problems associated with the compilation of a detailed dietary history from interviewing patients with anorexia nervosa. The following points were discussed.

1. Reliability of dietary information obtained from the patient. In general, it was found that this could be divided into the following categories. (a) Information of questionable reliability readily given (patients who were vomiters). (b) Information difficult to obtain (patients who were abstainers). (c) Information reliably and frankly given (patients exhibiting abstention interspersed with bulimic episodes). On the basis of these three groups, detailed dietary histories were obtained, selecting one example from each group.

2. Techniques for obtaining dietary histories and their bearing on, and interpretation of, recorded information. Dietary histories were taken by the same dietitian, so that although these were interpretations by the dietetic interviewer as to actual quantities of foods allegedly eaten, there was consistency in interpretation (as regards bias).

3. Factors which may be considered general characteristics of the illness regardless of the group to which the patient belonged, for example carbohydrate deprivation (Crisp, 1965, 1967). Patients often associated 'distention and fullness' with the eating of carbohydrate foods, although the patient's concept of carbohydrate foods is often confused, e.g., failure to appreciate that fruits and fruit juices contain carbohydrate.

4. Attitudes towards refeeding under the hospital dietary treatment regime; the order in which foods become re-accepted, with high-carbohydrate foods like potatoes, bread and cakes being taken late in treatment.

## REFERENCES

- Crisp, A. H. (1965). *Proc. R. Soc. Med.* **58**, 814.  
 Crisp, A. H. (1967). *J. psychosom. Res.* **11**, no. 1. (In the Press.)

**Anorexia nervosa and serum cholesterol levels.** By L. M. BLENDIS  
 (introduced by G. L. S. PAWAN), *The Middlesex Hospital, London, W1*

Although earlier workers reported normal or low serum cholesterol levels in patients with anorexia nervosa, in the last 2 years there have been reports of raised serum cholesterol levels in these patients (Crisp, 1965; Oberdisse, Solbach & Zimmerman, 1965; Klinefelter, 1965).

In our series of thirty-four female patients, although the serum cholesterol levels varied from 120 to 500 mg/100 ml, they were, as a group, very significantly higher than those of normal subjects matched individually for age.

Eight consecutive patients with anorexia nervosa admitted into hospital during the last 2 years were studied from the point of view of their initial serum cholesterol levels and the change in these levels during treatment. All patients were treated identically by means of bed rest, increasing doses of chlorpromazine, high-calorie diets and supportive psychotherapy (Crisp, 1966). Three patterns of change in serum cholesterol levels in these patients as they steadily gained weight emerged: (1) the serum cholesterol levels steadily rose; (2) serum cholesterol levels steadily decreased; (3) serum cholesterol levels remained unchanged.

Possible aetiological factors for both the initial serum cholesterol levels and for the different patterns of change during treatment are discussed. These include the effects of starvation and semi-starvation, patterns of eating, endocrine abnormalities and psychopathology.

## REFERENCES

- Crisp, A. H. (1965). *J. psychosom. Res.* **9**, 67.  
 Crisp, A. H. (1966). *Br. J. Psychiat.* **112**, 505.  
 Klinefelter, H. F. (1965). *J. clin. Endocrin.* **25**, 1520.  
 Oberdisse, K., Solbach, H. G. & Zimmerman, H. (1965). In *Anorexia Nervosa, Göttingen Symposium*, p. 26. [J. E. Meyer and H. Feldmann, editors.] Stuttgart: Georg. Thieme Verlag.

**Fat metabolism in anorexia nervosa.** By G. L. S. PAWAN, *Department of Medicine and Institute of Clinical Research*, and A. H. CRISP and L. M. BLENDIS, *Academic Unit of Psychiatry, Middlesex Hospital, London, W1*

It has been shown (Crisp, 1965; Blendis & Crisp, 1967) that a high blood chole-

sterol level is a characteristic feature in certain patients with anorexia nervosa. A study was therefore made of some other indices of fat metabolism in these patients.

At intervals throughout treatment on the regime described by Blendis & Crisp (1967), blood samples were obtained after an overnight fast and 24-hourly urine collections made. Blood plasma total lipids (Swahn, 1952), cholesterol (Zurkowski, 1964), triglycerides (Van Handel, 1961), lipid phosphorus (Youngburg & Youngburg, 1930), ketones (Pawan, 1958), free fatty acids (Dole & Meinertz, 1960), and 24-hourly urine FMS (Kekwick & Pawan, 1967), were determined.

In the early stages of treatment, the group of patients with elevated plasma cholesterol levels showed a tendency to exhibit raised plasma triglycerides, ketones and phospholipids with normal free fatty acids, whereas the patients with normal cholesterol levels had normal triglycerides, phospholipids and low free fatty acid concentrations in blood. As treatment progressed, these lipid parameters exhibited striking lability in their concentrations which appeared to be unrelated to variations in caloric intake or to the qualitative composition of the diet. It is possible, but by no means certain, that these changes in blood lipids may be related to variations in emotional state of the patients (Bogdonoff, 1960; Klein, Troyer, Back, Hood & Bogdonoff, 1965).

Assay of FMS activity in the urine, at intervals throughout treatment, indicated that this condition, in general, is not associated with high titres of FMS, e.g., compared with that seen in normal and lean subjects fasted for 1–2 days (Chalmers, Pawan & Kekwick, 1960) or in some obese patients fasted for 1–10 days (Kekwick & Pawan, 1967). In some of the patients urinary FMS activity was evident on admission; these patients showed evidence of overeating and vomiting, sometimes in secret and denied. In other patients 24 h urine samples soon after admission failed to reveal any FMS activity, but later in treatment, FMS appeared in the urine, was detectable for 2–4 weeks and subsequently completely disappeared as weight gain occurred with improved nutritional status.

Our thanks are due to Miss Sylvia Godfrey for skilled technical assistance.

#### REFERENCES

- Blendis, L. M. & Crisp, A. H. (1967). (In the Press.)  
Bogdonoff, M. D. (1960). *Archs intern. Med.* **105**, 505.  
Chalmers, T. M., Pawan, G. L. S. & Kekwick, A. (1960). *Lancet* *ii*, 6.  
Crisp, A. H. (1965). *Proc. R. Soc. Med.* **58**, 814.  
Dole, V. P. & Meinertz, H. (1960). *J. biol. Chem.* **235**, 2595.  
Kekwick, A. & Pawan, G. L. S. (1967). *Metabolism* (In the Press.)  
Klein, R. F., Troyer, W. G., Back, K. W., Hood, T. C. & Bogdonoff, M. D. (1965). *Metabolism* **14**, 17  
Pawan, G. L. S. (1958). *Biochem. J.* **68**, 33p.  
Swahn, B. (1952). *Scand. J. clin. Lab. Invest.* **4**, 98.  
Van Handel, E. (1961). *Clin. Chem.* **7**, 249.  
Youngburg, G. E. & Youngburg, M. V. (1930). *J. Lab. clin. Med.* **16**, 158.  
Zurkowski, P. (1964). *Clin. Chem.* **10**, 451.

**Some effects of cigarette smoke on guinea-pigs.** By J. R. EVANS, R. E. HUGHES and P. R. JONES, *Welsh College of Advanced Technology, Cardiff*

There are several indications in the literature that cigarette smokers have lowered tissue levels of vitamin C (Larson, Haag & Silvette, 1961; Calder, Curtis & Fore, 1963). Experiments were designed to investigate this using growing guinea-pigs exposed under strictly controlled conditions to cigarette smoke.

Two groups of fifteen male guinea-pigs with a mean initial body-weight of 350 g were used. They were dosed orally with ascorbic acid (0.2 mg/100 g body-weight daily) and for two 10 min periods each day one of the groups breathed the cigarette smoke. The second group was a control one. The smoke was produced by burning cigarettes in a standard smoking machine fitted with adaptors. This produced a smoke-air mixture similar in composition to that believed to be present in the respiratory passages of a human smoker during the act of smoking.

After 28 days the animals were killed and the tissue vitamin C levels determined. There was no significant difference between the testis and liver concentrations in the two groups but the levels in the adrenal glands were significantly different. The concentration in the 'smokers' adrenals was  $22.0 \pm 7.9$  mg/100 g (group mean with standard deviation) and in the non-smokers,  $31.1 \pm 5.4$ .

The 'smokers' gained weight less rapidly than the control group; after 28 days their mean body-weight was 419 g compared with 599 g for the controls. During the first 3 weeks of the experiment the reduced growth rate of the 'smokers' could not be attributed to any corresponding reduction in their food intake. After the 3rd week, however, the food intake of the 'smokers' fell off suddenly and this was paralleled by a still more marked depression of growth.

This work was made possible by a grant from the Beecham Food and Drink Division. The cigarettes were a gift from the Tobacco Research Council.

#### REFERENCES

- Calder, J. H., Curtis, R. C. & Fore, H. (1963). *Lancet* i, 556.  
Larson, P. S., Haag, H. B. & Silvette, H. (1961). *Tobacco, Experimental and Clinical Studies*, p. 336. Baltimore: The Williams and Wilkins Company.

**Interrelationships of the protein, energy and food intake of sows with the vitamin A, nitrogen and wet weight of newborn piglet livers.**

By D. L. FRAPE, R. W. HOCKEN, K. WOLF and L. G. CHUBB, *Spillers Limited, Animal Nutrition Research Laboratory, Middle Aston House, Nr Steeple Aston, Oxford*

It has been shown that there is an increased retention of vitamin A by chick livers but a drop in plasma albumin when the chick's protein intake is lowered (Nir & Ascarelli, 1966). Nevertheless Vakil, Roels & Trout (1964) consider that low dietary protein depresses absorption in rats to a greater extent than it interferes with its utilization, but evidence concerning the effect of protein quality is conflicting

(Quarterman & Saraiva, 1965; Esh & Bhattachanga, 1960). A general increase in the number of litters per sow per year coupled with a reduction in gestation food and protein intake has led to speculation that vitamin A could be limiting in the dietary of breeding sows. Furthermore it was considered that variations in protein consumption may influence the N and vitamin A liver reserves of their piglets at birth. Accordingly an experiment was designed for which breeding gilts were selected.

The treatments were designated HH, LL, HL, and LH, where the first letter of each treatment represents the gestation protein level (H=high, L=low) and the second the level of gestation food intake (again H=high and L=low). During lactation the intake levels were switched but all females were fed the high-protein diet. Both diets contained approximately the same concentrations of vitamin A. After a 2-year period the energy content of both diets was raised by about 8% and the protein quality improved, but its concentration decreased.

Over 200 small piglets were investigated at birth. Significant effects of treatment were observed during the first 2 years on wet liver weights and their vitamin A contents, and the estimated liver vitamin A reserve in the litter. Significant relationships between the various characteristics within treatment were also found. Some of the results found in the first period are given in Table 1. There was no change

Table 1. *Treatment mean piglet and liver weights (g) and liver vitamin A content (mg) during the period of low-energy dietary (period 1)*

Treatment	Piglet* weight	Fresh liver weight	Liver vitamin A		
			Per piglet analysed	Estimated per litter	Estimated 100 × liver vitamin A/ feed vitamin A
LL	939	34.5	0.587	7.7	2.3
HH	992	41.0	1.017	15.3	3.6
LH	935	33.0	0.769	9.5	2.2
HL	985	39.8	0.915	10.2	2.6
SE of treatment means		±2.55	±0.068	±1.2	

\*Mean of slaughtered piglets.

in the ratio 100 × liver vitamin A/feed vitamin A when the energy content and the protein quality of the diets were changed, and there was no obvious decline in the ratio with age of sow. However the previous treatment effects were not observed following the alteration in the diets.

Despite there being a relatively low apparent ratio of liver to feed vitamin A it is concluded that the concentration of the dam's dietary protein and her food intake level both influence the quantity of liver vitamin A in the newborn when the energy intake is minimal. Increased dietary protein appeared to be a more potent factor than increased food intake in raising vitamin A storage.

#### REFERENCES

- Esh, G. C. & Bhattachanga, R. K. (1960). *Symposium on Proteins*, p. 107. Mysore, India: Central Food Technological Research Institute.  
 Nir, I. & Ascarelli, I. (1966). *Br. J. Nutr.* **20**, 41.  
 Quarterman, J. & Saraiva, G. C. (1965). *Proc. Nutr. Soc.* **24**, xx.  
 Wakil, U. K., Roels, O. A. & Trout, M. (1964). *Br. J. Nutr.* **18**, 217.