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Effects of Caecectomy in the Young Adult Female Rat on Digestibility of Food Offered *ad libitum* and in Restricted Amounts

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Abstract

Caecectomized and sham-operated rats were fed a laboratory chow *ad libitum* and the effects of caecectomy on the digestibility of the food were studied. Compared with sham-operated controls, caecectomized rats showed a decrease in apparent digestibility of organic matter from 77·8 to 73·0%, of crude protein from 83·0 to 79·4%, and of 'carbohydrate' from 74·6 to 69·0%. However, faecal water content increased from 41·6 to 54·8%. ^{51}Cr-labelled EDTA was excreted faster in the faeces after caecectomy. The colon partly adapted to the loss of caecal mucosa by increased length and thus mucosal surface area.

In a second concurrent experiment the effect of caecectomy on the apparent digestibility of food during food restriction was studied. Six caecectomized rats, comparable in all respects to those used in the first study, were fed the laboratory chow *ad libitum* for 3 weeks. They were then fed submaintenance amounts of food to achieve body weight losses of 40–50% and to maintain these low weights for 4 weeks. Finally, they were again fed *ad libitum* for 3 weeks. During the period of restriction the apparent digestibility of organic matter increased from 72·7 to 75·4%. This was largely due to the increased apparent digestibility of crude protein which rose from 78·4 to 81·9%. Digestibility coefficients returned to control values immediately upon refeeding *ad libitum*.
[Other keywords: digesta flow, colon histology.]

Introduction

Submaintenance intake of nutritionally adequate food by the rat enhances the rates of absorption of D(+)glucose and some amino acids from the small intestine (Kershaw *et al.* 1960; Lis *et al.* 1972; Williams *et al.* 1976). Williams and Senior (1978) showed that prolonged restricted intake of a laboratory chow by young adult rats, which caused 40–50% body weight loss, resulted in increased digestibility of crude protein but the coefficients for lipid and carbohydrate were not affected. The present paper includes data from an experiment which showed that the change in digestibility of crude protein was not dependent on the caecum.

A study was also made of the effects of caecectomy in the rat on digestibility of a laboratory chow fed *ad libitum*, the rate of passage of ^{51}Cr-labelled EDTA through the digestive tract, and the anatomy and histology of the ileum and colon.

Materials and Methods

Animals

Young adult nulliparous rats derived from the Sprague–Dawley strain were used. The animals were all from a special mating with a maximum difference in littering time of 3 days. As the animals were not individually identified at this stage they were all considered to be 47 days of age and to weigh 132±2·9 g (mean ±s.e.).

Housing and Environmental Conditions

All animals were housed individually in wire mesh metabolism cages. The environmental temperature ranged from 21 to 25°C and the photoperiod was 12 h light from 0600 to 1800 h.

Feeding, Collection of Faeces and Weighing of Animals

A standard laboratory chow (Fielders Pty Ltd, Tamworth, N.S.W.), fed in ground form, was used. It contained 82·4% grain and grain derivatives, 13% fish plus meat meals, 4% lucerne meal, 0·3% sodium chloride and 0·3% vitamin supplement. Proximate analysis showed it contained 6·8% ash, 24·8% crude protein (N×6·25), 5·1% lipid and 63·3% 'carbohydrate'; the latter was calculated by difference. The gross energy content was 19 kJ/g. Further details are given by Williams *et al.* (1976). During *ad libitum* feeding the animals were provided with weighed excess each day and the amount consumed calculated from any remainder. For restricted feeding a known daily quantity was offered; usually there was no residue.

The gut was allowed to equilibrate to any new quantity of food supplied during the first 2 days of each weekly period. Faeces were then collected each day for the next 5 days, dried at 97°C for 24 h and bulked. The bulked faeces were then dried for a further 24 h before measurement of total dry matter.

The rats were weighed before feeding on the first day of each week.

Chemical Analysis

Measurements of faecal and food lipid were made by light petroleum (b.p. 40–70°C) extraction of ground samples for 24 h in a Soxhlet apparatus and calculated as weight loss. Nitrogen contents were obtained by the Kjeldahl technique using selenium as the catalyst. Ash was measured on dried ground samples by oxidation in a muffle furnace at 400°C for 1 h followed by 18 h at 600°C.

Rate of Passage of Digesta

The rate of passage was determined by starving the rats overnight and then administering ^{51}Cr-labelled EDTA of 200 000 cpm nominal activity by stomach tube between 0950 and 1010 h. The animals were offered food *ad libitum* immediately after ^{51}Cr-labelled EDTA administration. Faeces were collected at 3-h intervals until 1900 h then at 2-h intervals until 1000 h the next day. The radioactivity of dried faeces was determined by gamma counting in a Packard Tri-carb scintillation spectrometer (model 3002) fitted with a sodium iodide–thallium-activated crystal. Correction was made for decay in the interval between collection and counting.

Faecal Water Content

This was determined by weighing faeces immediately after collection at intervals of 2 or 3 h, drying for 24 h at 97°C and reweighing.

Caecectomy

Rats were starved for 12 h before surgery. Anaesthetic was administered by the method of Mauderly (1975), but a separate induction jar was used. The anaesthetic mixture was halothane in equal parts of nitrous oxide and oxygen.

Laparotomy was performed on the left abdominal flank. No haemostasis was required. The caecum was brought out through a gauze swab. In the sham-operated animals it was immediately replaced in the body cavity. In the animals to be caecectomized a simple ligature was placed at the base of the caecum and tightened. Care was taken to ensure that a passageway for digesta was retained between the ileum and colon. The caecum was clamped off close to the ligature with a haemostat and it was then excised. The exposed mucosa of the caecum was cleansed with saline and treated with Neotracin antibiotic spray (Ethnor Pty Ltd, Sydney, N.S.W.). The colon was then replaced within the body cavity, the muscle layers sutured and the skin incision closed using silk. The area was coated with Neotracin soluble dusting powder (Ethnor Pty Ltd, Sydney, N.S.W.) and sprayed with Nobecutane (B.D.H. Pharmaceuticals Ltd, London, U.K.). All animals ate and drank within a short time after recovery from anaesthesia. However, most of them took a few days to resume more normal food intakes. Of the original 24 animals one was lost due to an overdose of anaesthetic, and another two did not recover as a result of blockage of the ileo–colonic passageway.

Anatomy and Histology

Anatomy

All animals were without food but had access to water for 24 h before being killed with an overdose of sodium pentobarbitone. After body weights had been measured a midline incision was made in the abdominal wall from the anus to the base of the neck. The small intestine was severed at the pylorus and the ileo–caecal junction and perfused *in situ* with formol saline. It was then carefully stripped from the mesentery and blotted dry on filter paper. The length of the small intestine was measured while it was hanging straight against a metre rule. It was then weighed. Finally, a specimen of ileum was obtained for later histological examination.

The pelvic girdle was split with scissors, the anal sphincters removed and the colon perfused with formol saline. It was then removed from the mesentery and measured for the same parameters as the small intestine.

Histology

The specimens of ileum and colon were fixed for 24 h in formol saline. They were post-fixed and dehydrated with Clark's mixture, stained with benzene–methylene green (Senior 1969) and embedded in paraffin wax (m.p. 54–56°C).

Transverse sections of the ileum and colon were cut at 8 μm and stained with Greenstein's stain (Greenstein 1961) and by Periodic acid–Schiff–Haemalum–Aurantia (Marks and Drysdale 1957). Measurements made on the ileum were maximum villus tip to serosal surface, villus height, crypt depth, muscle thickness and serosal circumference. The same measurements except villus height were made on the colon with the addition of mucosal circumference. Measurements were made of each parameter on six sections randomly selected from each specimen. The ileal serosal and colonic serosal and mucosal circumferences were measured by outlining the image of a section on tracing paper laid on the ground-glass focusing screen of a microscope camera and measuring its length with a map measurer. Absolute values were obtained by projecting a stage micrometer onto the same screen. Other measurements were made using a linear scale eyepiece graticule.

Statistical Analysis

Comparisons between means were made by Student's *t*-test unless otherwise indicated. Calculations of regression equations and comparisons of regression coefficients were made by standard procedures (Brownlee 1949). All means are presented with their standard errors.

Experimental

Effects of Caecectomy on Rats Fed ad libitum

Nine sham-operated and six caecectomized rats were used during a 17-week experiment to study food intakes, digestibility of the food, rate of passage of digesta and the amount of water in the faeces while food was offered *ad libitum*.

The moisture content of the faeces was determined during the rate of passage study, and also on one previous occasion by collecting faeces from 0900 h until 0900 h the next day at 3-h intervals.

On completion of the rate of passage study the animals were killed for anatomical and histological measurements of the small intestine and colon.

Effect of Restricted Food Intake on Digestibility in Caecectomized Rats

Concurrent with the above experiment, in the same room and using food from the same source, six caecectomized rats were fed *ad libitum* for 3 weeks; they were then fed 50% of their average daily *ad libitum* intakes for 7 days and during the following 6 weeks food supplied to each rat was further adjusted at weekly intervals to obtain a 50% loss in body weight by the end of this time. The rats were then fed appropriate amounts of food to prevent further change in body weight during the next 4 weeks. Finally, they were fed *ad libitum* once again for 3 weeks. Faeces were collected for analysis on the last 5 days of each weekly period throughout the entire 17 weeks.

Results

Effects of Caecectomy on Rats Fed ad libitum

Food intake

The caecectomized rats ate more than the sham-operated rats. The mean intakes of dry matter per day for the 17 weeks of the experiment were $15\cdot9\pm0\cdot2$ g for the caecectomized rats and $14\cdot7\pm0\cdot1$ g for sham-operated rats; the difference was significant ($P<0\cdot001$). As the mean body weights for the 17 weeks were $232\cdot8\pm2\cdot3$ and $229\cdot4\pm2\cdot7$ g for the sham-operated and caecectomized rats respectively, none of the difference was due to body weight.

Body weight

Both caecectomized and sham-operated rats grew satisfactorily after recovery from surgery. The initial mean body weights when digestibility studies commenced and final mean body weights were $187\cdot0\pm5\cdot8$ and $255\cdot0\pm11\cdot4$ g respectively for the caecectomized rats and $199\cdot0\pm6\cdot8$ and $255\cdot0\pm8\cdot7$ g respectively for the sham-operated animals.

Digestibility of food and its constituents

The digestibility coefficients did not consistently alter with increase in age in either group of animals; therefore, mean values are presented. The effect of caecectomy was to decrease the digestibility coefficients for dry matter and organic matter compared with the sham-operated rats. The means for the 17 weeks of the experiment were $70\cdot2\pm0\cdot2$ *v.* $74\cdot6\pm0\cdot1$% ($P<0\cdot001$) for dry matter and $73\cdot0\pm0\cdot02$ *v.* $77\cdot8\pm0\cdot1$% ($P<0\cdot001$) for organic matter. These differences were partly due to the apparent digestibility of crude protein which was lower after caecectomy ($79\cdot4\pm0\cdot2$ *v.* $83\cdot0\pm0\cdot1$%, $P<0\cdot001$), and partly to 'carbohydrate' ($69\cdot0\pm0\cdot3$ *v.* $74\cdot6\pm0\cdot2$%, $P<0\cdot001$). There was no detectable difference in digestibility of lipid.

Rate of passage of non-absorbable marker through the gut

Marker was detected in the faeces of both groups of rats during the second 3-h collection. However, the time of maximum concentration of marker in the faeces was variable within animals within both groups. After peak concentration was attained it decreased with time. Regression equations were calculated from data obtained from collections between 12 and 24 h after giving the marker. The equation for the sham-operated rats was

$$\log y_1 = 7\cdot6-2\cdot76 \log x, \qquad r = 0\cdot67, \qquad P<0\cdot001;$$

and for the caecectomized rats it was

$$\log y_2 = 15\cdot0-9\cdot64 \log x, \qquad r = 0\cdot87, \qquad P<0\cdot001,$$

where y is marker concentration (in counts per minute per gram of faeces), and x is time (in hours). The regression coefficients were significantly different ($P<0\cdot001$). Thus the marker concentration in the faeces decreased substantially faster in caecectomized rats than in sham-operated animals.

Water content of the faeces

The water content was higher in the faeces of caecectomized rats (54·8±2·8%) than in the faeces of the sham-operated rats (41·6±1·0%) ($P<0\cdot001$).

Anatomy and histology

The values for gross anatomical parameters for the small intestine are presented in Table 1. There were no significant differences between sham-operated and caecectomized animals. Differences in histological measurements on the ileum were also not significant; they have not been presented.

Data for the colon are also shown in Table 1. Wet weight ($P<0\cdot01$) and length ($P<0\cdot001$) were statistically greater in the caecectomized animals than in the sham-operated rats. Mean mucosal circumference was not significantly different in the two groups but because of the greater length of colon the mucosal surface area was greater in the caecectomized than in the sham-operated animals ($P<0\cdot01$).

Table 1. Anatomy of the ileum and colon in sham-operated and caecectomized rats fed *ad libitum*
Values given are means ±s.e.; n.s., not significant ($P>0\cdot05$)

Organ	Parameter	Sham-operated	Caecectomized	P
Whole body	Body weight (g)	235·0±12·4	236·0±10·6	n.s.
Small intestine	Wet weight (g)	5·60±0·37	5·90±0·32	n.s.
	Length (cm)	103·0±3·9	104·0±3·7	n.s.
	Wet weight (g/cm)	0·050±0·002	0·050±0·002	n.s.
Colon	Wet weight (g)	1·70±0·09	2·40±0·16	0·01
	Length (cm)	17·4±0·4	21·7±0·6	0·001
	Wet weight (g/cm)	0·100±0·003	0·110±0·006	n.s.
	Muscle thickness (μm)	88±4	106±34	n.s.
	Crypt depth (μm)	200±18	263±31	n.s.
	Mucosal circumference (mm)	10·6±0·5	12·4±0·8	n.s.
	Mucosal surface area (mm^2)	1839·0±104·4	2688·0±203·0	0·01

Effect of Restricted Intake on Digestibility in Caecetomized Rats

Food intake

The results are shown in Fig. 1. When refed *ad libitum* after 11 weeks on below-maintenance food intake the rats ate more food per day (18·80±0·02 g) than when fed *ad libitum* before deprivation (15·90±0·03 g) ($P<0\cdot001$).

Digestibility of food and its constituents

Fig. 2 shows the apparent digestibility coefficients for organic matter and for crude protein. Statistical comparisons by Student's *t*-test were made between the mean coefficients for each animal during *ad libitum* food intake (weeks 1, 2, 3, 15, 16, 17) and after 40–50% body weight loss through restricted food intake (weeks 8–14 inclusive). Because the differences were not significant for all animals, comparisons were also made by a paired *t*-test using the means for each animal, i.e. between overall treatment means.

For organic matter, four of the six animals had significantly greater digestibility coefficients during restricted feeding than during *ad libitum* food intake ($P<0\cdot01$,

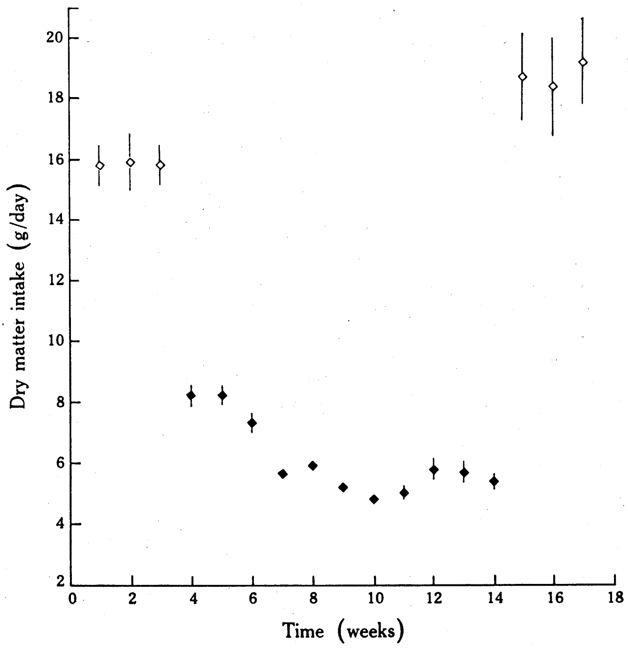

Fig. 1. Means and standard errors of the dry matter intakes of caecectomized rats.
◊ *Ad libitum* food intake.
◆ Restricted food intake.

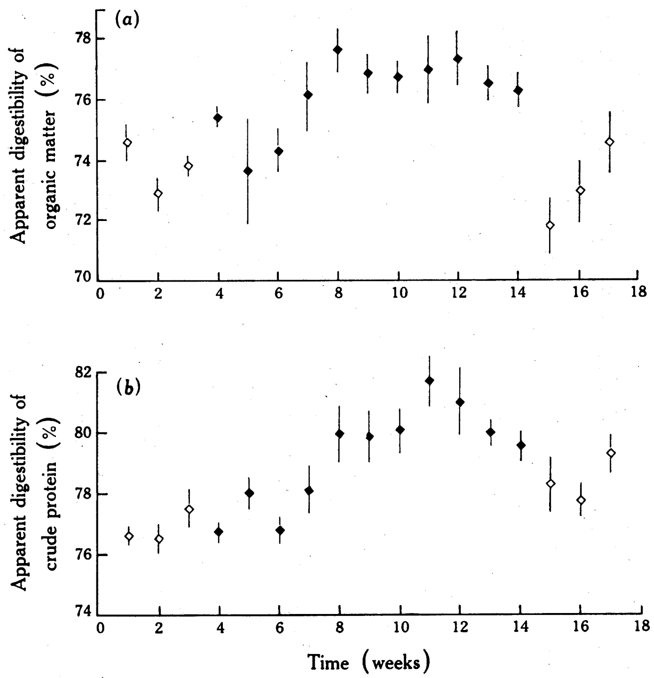

Fig. 2. Mean apparent digestibility coefficients and standard errors for (*a*) organic matter and (*b*) crude protein in caecectomized rats fed *ad libitum* (◊) and during restricted food intake (◆).

0·01, 0·001, 0·001) and the overall treatment means were significantly different ($75 \cdot 4 \pm 0 \cdot 3$ *v.* $72 \cdot 7 \pm 0 \cdot 4\%$, $P < 0 \cdot 02$).

Generally, the results for apparent digestibility of crude protein showed the same pattern as the results for organic matter. Five (all $P < 0 \cdot 01$) of the six animals had higher mean digestibility coefficients during restricted than during *ad libitum* food intake. The overall treatment means were also significantly different ($81 \cdot 9 \pm 0 \cdot 2$ *v.* $78 \cdot 4 \pm 0 \cdot 4\%$, $P < 0 \cdot 001$).

Three of the six rats had significantly greater digestibility of 'carbohydrate' during restriction ($P < 0 \cdot 05$, 0·01, 0·01), but the overall mean values of $69 \cdot 0 \pm 0 \cdot 4$ and $71 \cdot 6 \pm 0 \cdot 4\%$ for *ad libitum* and restricted food intakes respectively were not significantly different. Consequently the results must be considered equivocal.

Four of the six rats had significantly greater digestibility for lipid during restriction ($P < 0 \cdot 05$, 0·05, 0·01, 0·01), and the overall mean values of $90 \cdot 5 \pm 0 \cdot 3$ and $93 \cdot 4 \pm 0 \cdot 3\%$ for *ad libitum* and restricted food intakes respectively were also different ($P < 0 \cdot 001$).

Discussion

Effects of Caecectomy in Rats Fed ad libitum

Caecectomized rats had greater food intakes than sham-operated rats; this was almost certainly a response to the lower digestibility of organic matter in these rats. Non-ruminant animals usually eat to obtain energy requirements as shown by appetite for high bulk, poorly digestible food (Dowling *et al.* 1967) and the hyperphagia of lactation (Cripps and Williams 1975). However, in another experiment using a different strain of rat (Williams and Senior, unpublished data), food intake was not increased by caecectomy which suggests that the sensitivity of the hypothalamic nuclei controlling food intake can vary with rat strain. Further, in these animals a difference in dry matter digestibility was produced by caecectomy comparable to that reported in this paper. Consequently, the effect of caecectomy on food digestibility reported in this paper was presumably not influenced by the greater food intake.

Dreyer *et al.* (1975) studied the effect of caecectomy in the rat, and found the digestibility of dry matter by intact and caecectomized animals to be $99 \cdot 0 \pm 1 \cdot 7$ and $90 \cdot 0 \pm 2 \cdot 2\%$ (mean ± s.e.) respectively; in the present experiment, with a less digestible food, there was a smaller but significant decrease due to caecectomy ($74 \cdot 6 \pm 0 \cdot 1$ *v.* $70 \cdot 2 \pm 0 \cdot 2\%$). The former workers also concluded that caecectomy did not affect the digestibility of crude protein, but in the present experiment the digestibility of this nutrient was significantly reduced; the different compositions of the foods used may have been responsible for the different responses obtained.

Yang *et al.* (1969) showed that excision of the caecum decreased the digestibility of acid detergent-treated fibre in rats. As the food used in the present study contained an estimated 5% crude fibre it is not surprising that caecectomy decreased the digestibility of 'carbohydrate'.

Caecectomy in the rat removes the major pool for mixing and retention of digesta in the gut distal to the stomach; this was reflected in the faster rate of passage of a non-digestible fluid marker through the intestines. The technique used did not allow a separate measurement of transit rate in the small intestine; it may have been increased. If this did occur some of the difference in digestibility between the caecectomized and sham-operated rats could have been due to a decrease in available time for enzymic digestion in the small intestine. Iwai *et al.* (1973) showed in mice, with variable caecal size per unit body weight produced by manipulation of the gut microbial flora, that the transit rate of chromium sesquioxide through the small intestines was slower the bigger the caecum; however, it is not possible to state with certainty that the size of the caeca caused the different rates.

The effect of caecectomy on the degree of coprophagy in the rat has not been determined. It is therefore possible that the increased rate of passage of marker in caecectomized animals could have been due in part to decreased coprophagy.

As the large intestine is a major site of absorption of water, decrease in available surface area due to caecectomy no doubt accounted for much of the increase in faecal water. Scarpello *et al.* (1978) caecectomized rats, but their technique involved excision of the terminal 2–3 cm of ileum; thus they removed the ileo–caecal valve. Their animals had diarrhoea for up to 2–3 weeks after surgery; this was not shown by caecectomized rats in the present experiment.

Caecectomy caused an increase in the surface area of the colonic mucosa—an appropriate adaptation; this was due to an increase in length rather than an increase in mucosal circumference. No effect of caecectomy on weight per unit length of colon was shown; Scarpello *et al.* (1978) also failed to show an influence of caecectomy on this parameter.

It can be concluded that caecectomy decreased the apparent digestibility of 'carbohydrate' and crude protein and caused an increase in mucosal surface area of the colon which partly compensated for the loss of caecal mucosa.

Effect of Restricted Intake on Digestibility in Caecectomized Rats

This part of the study showed that the main effect on digestibility of restricting the food intake of caecectomized rats was to increase the apparent digestibility of crude protein; Williams and Senior (1978) showed a similar effect in intact rats. Loesche (1968), from a comparison between conventional and germ-free rats, showed that microbial activity in the caecum caused greater absorption of nitrogen from the digesta, presumably as ammonia. The results now presented show that the increased apparent digestibility of crude protein produced by food restriction in intact rats (Williams and Senior 1978) was not due to proportionately greater caecal microbial fermentation of protein and absorption of the products.

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References

Brownlee, K. A. (1949). 'Industrial Experimentation'. 4th edn. (H.M.S.O.: London.)

Cripps, A. W., and Williams, V. J. (1975). The effect of pregnancy and lactation on food intake, gastrointestinal anatomy and the absorptive capacity of the small intestine in the albino rat. *Br. J. Nutr.* **33**, 17–32.

Dowling, R. H., Riecken, E. O., Laws, J. W., and Booth, C. C. (1967). The intestinal response to high bulk feeding in the rat. *Clin. Sci.* **32**, 1–9.

Dreyer, J. J., Concannon, T. R. J., and de Klerk, W. A. (1975). Digestibility of protein and dry matter in caecectomized and intact rats. *S. Afr. Med. J.* **49**, 1661–6.

Greenstein, J. S. (1961). A simplified five dye stain for sections and smears. *Stain Technol.* **36**, 87–8.

Iwai, H., Ishihara, Y., Yamanaka, J., and Ito, T. (1973). Effects of bacterial flora on cecal size and transit rate of intestinal contents in mice. *Jpn J. Exp. Med.* **43**, 297–305.

Kershaw, T. G., Neame, K. D., and Wiseman, G. (1960). The effect of semi-starvation on absorption by the rat small intestine *in vitro* and *in vivo*. *J. Physiol.* (*London*) **152**, 182–90.

Lis, M. T., Crampton, R. F., and Matthews, D. M. (1972). Effect of dietary changes on intestinal absorption of L-methionine and L-methionyl-L-methionine in the rat. *Br. J. Nutr.* **27**, 159–67.

Loesche, W. J. (1968). Accumulation of endogenous protein in the caecum of the germfree rat. *Proc. Soc. Exp. Biol. Med.* **129**, 380–4.

Marks, I. N., and Drysdale, K. M. (1957). A modification of Zimmermann's method for differential staining of gastric mucosa. *Stain Technol.* **32**, 48.

Mauderly, J. L. (1975). An anaesthetic system for small laboratory animals. *Lab. Anim. Sci.* **25**, 331–3.

Scarpello, J. H. B., Cary, B. A., and Sladen, G. A. (1978). Effects of ileal and caecal resection on the colon of the rat. *Clin. Sci. Mol. Med.* **51**, 241–9.

Senior, W. (1969). Staining of animal tissues with the dye base of methylene green in benzene to facilitate identification and selection of material. *Stain Technol.* **44**, 269–71.

Williams, V. J., and Senior, W. (1978). The effect of semi-starvation on the digestibility of food in young adult female rats. *Aust. J. Biol. Sci.* **31**, 593–9.

Williams, V. J., Senior, W., and Sippel, J. (1976). Effects of semi-starvation on the total body composition and absorptive function of the small intestine of the young adult female rat. *Aust. J. Biol. Sci.* **29**, 341–9.

Yang, M. G., Manoharan, K., and Young, A. K. (1969). Influence and degradation of dietary cellulose in cecum of rats. *J. Nutr.* **97**, 260–4.

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