

CHANGES IN STRUCTURE AND FUNCTION OF THE RAT ALIMENTARY TRACT  
FOLLOWING REMOVAL OF THE COLON.

PETER CLEMENT MASESA.

Thesis submitted for the degree of Ph.D.

UNIVERSITY OF EDINBURGH.

1975



# ABSTRACT OF THESIS

Name of Candidate .....PETER CLEMENT MASESA.....  
Address c/o Faculty of Medicine, University of Dar es Salaam, P.O.Box.20693,  
Dar es Salaam. TANZANIA.  
Degree .....Doctor.....of.....Philosophy..... Date .....9th September, 1975.....  
Title of Thesis .....CHANGES IN STRUCTURE AND FUNCTION OF THE RAT ALIMENTARY TRACT  
.....FOLLOWING REMOVAL OF THE COLON.....

1. Observed over a period of 2-4 months after operation, adult rats which have been subjected to either right hemicolectomy (caecectomy), left hemicolectomy, subtotal colectomy or subtotal colon bypass grow nearly as fast as both intact rats and rats which have undergone terminal ileum transection and anastomosis.
2. Although diarrhoea is a prominent feature during the first two to three weeks after caecectomy, and during the first three to four weeks after subtotal colectomy or colon bypass, measurement of energy equivalent of the food ingested and the faeces failed to indicate marked malabsorption three months after subtotal colectomy.
3. The transit of barium sulphate through the small intestine is markedly reduced in caecectomised rats, but both the stomach and small bowel emptying times are greatly prolonged; the major portion of the meal appears to stagnate in the lower part of the small intestine. Subtotal colectomy, in which less than 5cm of terminal large bowel (mainly rectum) remains, shortens the stomach-to-anus transit by 2-4 hours.
4. Examination of tail blood indicated that anaemia does not develop after subtotal colectomy, or at least not during the first three **post-operative months**; the blood picture was normal at 2, 4 and 12 weeks after surgery.
5. Although the colon is shorter in both caecectomised and left hemicolectomised rats, when measured per unit length it weighed 83% and 33% respectively, more than in controls. The increase in weight involves both the mucosa and seromuscular coat in right hemicolectomy, but is mainly confined to the mucosa in left hemicolectomy.
6. For animals of similar body weight, the small gut is approximately the same in length in rats subjected to right or left hemicolectomy, subtotal colectomy or colon bypass as in both intact rats and rats which have undergone sham operation. Whereas the weight of the small intestine in left hemicolectomy rats is similar to that in controls, that in caecectomised rats increases by 20-25%. The mucosa, rather than the seromuscular coat, is the site of the increase, which is more pronounced in the lower third of the intestine than in the upper and middle thirds. On the other hand, in rats subjected to subtotal colectomy or colon bypass,

*Use other side if necessary.*

the small intestine becomes about one third heavier than in controls; the major increase occurs in the mucosa, especially of the upper and lower thirds, although the seromuscular coat of the lower third is also heavier than in controls.

7. Following right hemicolectomy the villi do not increase in height, although the number of enterocytes sectioned in a unit length of villus mid-margin is 6-7% above control value all along the distal two thirds of the small gut. On the other hand, the villi in subtotal colectomised rats are 10-12% taller than control villi in about 40cm of the upper small gut and again in 10cm of terminal ileum. The number of enterocytes per unit length of villus mid-margin increases along the entire length of the intestine by about 7%.

8. The considerable changes in the small gut weight in rats subjected to subtotal colectomy or colon bypass can be attributed to the 33-40% increase in food intake seen in these animals, and to the consequent increase in intraluminal nutrition. But there may be other stimuli at work: after 3 weeks of pair feeding, during which the colectomised animals lost 4% of their body weight, their small guts were still about 17% larger than those of control rats.

These changes in structure and function of the alimentary tract after partial or subtotal colectomy are discussed in the text together with three hypotheses relating to the mechanism by which growth of gut remnant may be induced.

To my family.

Declaration.

This thesis was composed by the undersigned who was also responsible for the design and execution of the research work described herein. The project was carried out under the supervision of Dr. J.M. Forrester, of the department of Physiology, Faculty of Medicine, University of Edinburgh.

Signature

... Date 27/8/1975

---

ACKNOWLEDGEMENTS

My sincere thanks are due to Dr. J.M. Forrester for suggesting the project, assisting in the planning and execution of some of the work, introducing me to specialists in various research fields whose advice proved very valuable in my work; and for his criticism and expert advice on the written work.

I am also grateful to Professor W.E. Watson for the material, instruments and apparatus used in this work; to Mrs. J. Anderson for assisting me at operations; to Dr. W.A. Dewar for helping in the preparation of barium sulphate-marked food; to Mr. A. Mackenzie and Mr. D. Shirling for the analysis of samples; to Dr. S.H. Davies for the examination of blood samples; to Dr. I.W. Percy-Robb for advice on thin-layer chromatography; to Mrs. K. Grant and her Assistants for helping in the preparation of specimens for histology; to Mr. M.J. Campbell for advice on statistics, to Mr. W. Lawson for the photographic work and to many others who contributed to the success of this work in various ways.

I am indebted to the World Health Organization for granting me a fellowship to undertake this study, and for meeting the research expenses.

---

SUMMARY.

1. Young adult rats, 2 to 4 months old, were subjected to either right hemicolectomy, left hemicolectomy, subtotal colectomy or subtotal colon bypass and observed for 2 to 4 months after surgery; their postoperative bowel habit, faecal output, food intake, energy absorption, body weight, intestinal transit-time, blood picture, intestinal length, gut weight, villus height and enterocyte density were compared to those of sham operated and unoperated control rats.

2. All rats subjected to right hemicolectomy developed diarrhoea (passing semi-fluid stools), but only during the first two to three weeks after surgery; the faecal dry matter output increased by about 30%. Rats with subtotal colectomy or colon bypass had a more severe watery diarrhoea during the first three to four postoperative weeks. However, this was replaced by semi-solid stools six weeks after operation; the faecal dry matter output during ad libitum feeding condition was 50 to 60% above control value at 2 weeks after surgery, and during the third and fourth postoperative month. When pair fed to prevent their hyperphagia (Paragraph 3) subtotal colectomised rats had a faecal dry matter output only 36% above control value. Coprophagy still occurred in subtotal colectomised animals; this was indicated by the presence of free bile acids in the stomach contents during the third month after subtotal colectomy. Left hemicolectomised animals developed no diarrhoea and their faecal dry matter output was similar to that of controls.

3. The food intake of rats with right or left hemicolectomy was similar to that of sham operated and unoperated controls. On the other hand,

subtotal colectomised rats, and those with subtotal colon bypass, developed hyperphagia amounting to 33 to 40% above control intake.

4. Measurement of energy equivalent of the food ingested and the faeces failed to indicate marked malabsorption in subtotal colectomised animals.

5. Left hemicolectomy had no significant influence on the body weight; on the other hand, right hemicolectomised rats lost, during the first week after operation, an average of 12% of the preoperative body weight, while the loss in sham operated controls was about 6%. Thereafter both right hemicolectomised and sham operated rats gained weight at a similar rate, which was significantly greater than that in unoperated rats; but in spite of growing at a rate greater than normal, right hemicolectomised rats remained underweight for a considerable period, when compared to controls. The loss of weight in subtotal colectomised rats, at the end of the first week after operation, amounted to 13% of the preoperative weight. At four months after surgery these animals were still underweight when compared to controls, although the rate of body weight gain was approximately the same in sham operated and in the colectomised rat. Generally, animals operated on between the age of 2 and 3 months showed less marked postoperative body weight loss and subsequently maintained approximately the same body weight as unoperated controls, even after they had been subjected to subtotal colectomy.

6. Transit of barium sulphate through the small intestine was studied in 6 right hemicolectomised rats (three months postoperatively) and in 6 unoperated controls by x-ray examination of the unanaesthetised animal; transit-time was significantly reduced after right hemicolectomy. On



the other hand, the small bowel emptying time, as well as the stomach emptying time, was markedly prolonged in right hemicolectomised rats. The stomach-to-anus transit of a barium meal was studied in 12 rats before surgery and in 6 subtotal colectomised, and 6 unoperated, rats at three weeks and again at three months after operation, by collecting faeces at frequent intervals and examining them by radiography. Subtotal colectomy reduced the stomach-to-anus transit by 2 to 4 hours.

7. Examination of tail blood carried out in 8 subtotal colectomised and 8 sham operated rats at 2, 4 and 12 weeks after surgery failed to detect any abnormalities.

8. The length and weight of the large intestine were measured in 8 right hemicolectomised, 8 left hemicolectomised, and 16 sham operated rats three months after surgery. The colon (caecum excluded) was significantly shorter after either right or left hemicolectomy, even when the amount of colon removed at operation was added to the colon at killing. In spite of this, the colon in right hemicolectomised rats weighed 37% more than in control animals, and when measured per unit length the weight of the colon at killing was 83% heavier in operated than in control rats. In left hemicolectomised animals the total weight of the colon (i.e. amount of colon removed at operation added to colon at killing) was approximately the same as in control rats. But the colon was shorter in the hemicolectomised rat; when measured per unit length it weighed 33% more in the left hemicolectomy than in the control animal.

By weighing mucosa and seromuscular coat separately (after separation by scraping) it was found that the increase in colon weight involved both the mucosa and seromuscular coat in right hemicolectomy, but was mainly confined to the mucosa in left hemicolectomy.

9. The small intestine in rats subjected to right or left hemicolectomy, subtotal colectomy or colon bypass, and in sham operated or unoperated controls was approximately the same in length for animals of similar body weights.

10. The total weight for both the fresh and **dry** small gut was 20 to 25% heavier in right hemicolectomised rats than in either sham operated or unoperated controls. Although the small bowel increased in weight all along its length, the major change occurred in the lower third, where the location of weight increase was principally the mucosa. Left hemicolectomy had no influence on the weight of the small intestine.

11. Three months after subtotal colectomy or colon bypass the small bowel increased in weight by 33% when compared to that in control animals. The increase in weight was more marked in the upper and lower thirds, and was mainly confined to the mucosa, although the seromuscular coat of the lower third was also significantly heavier in operated than in control rats.

12. Villus height and enterocyte density was studied in 8 right hemicolectomised, 8 subtotal colectomised and 16 sham operated rats three months after surgery. Villus height measurements taken at 10 sites spaced at approximately equal length from the pylorus to the ileal end showed no change in villus size after right hemicolectomy, although enterocyte density along 150 $\mu$ m of mid-villus margin was increased. On the other hand, following subtotal colectomy the villi in the upper 40cm of small gut and in the 10cm of terminal ileum showed significant increase in size; and the number of enterocytes per 150 $\mu$ m of mid-villus edge was about 7% greater in the subtotal colectomy than in the control small gut.

13. Preliminary investigations on the absorption of nutrients from the intestinal tract of the unanaesthetised rat by whole animal balance studies with inert-marker were carried out. Barium sulphate was tested to find out<sup>1</sup> if it could be used as an inert indicator in the rat diet for rat studies. Results on recoveries of this marker, as estimated by X-ray Spectrometry, are discussed in part 5 of this thesis.

---

TABLE OF CONTENTS.PART 1.

Introduction and Historical review .....	page 1
--	--------

PART 2.

Plan of work .....	page 8
(a.i) Animal care .....	page 8
(a.ii) Allocation of animals to various experimental groups	page 9

Operative Procedures.

(b.i) Right Hemicolectomy .....	page 10
(b.ii) Left Hemicolectomy .....	page 11
(b.iii) Subtotal Colectomy .....	page 11
(b.iv) Subtotal Colon bypass.....	page 12
(b.v) Ileal Transection and Anastomosis (sham operation) .....	page 12

Measurement Procedures.

(c) Food Weighings .....	page 12
(d) Faecal weighings .....	page 13
(e) Intestinal Transit-time .....	page 14
(f) Energy absorption .....	page 15
(g) Blood sampling and examination .....	page 15
(h.i) Intestinal length measurement .....	page 16
(h.ii) Gut weight measurement .....	page 17
(h.iii) Mucosal separation from the seromuscular coat .....	page 17
(j.i) Preparation of microtome sections for histology .....	page 18
(j.ii) Villus height and enterocyte density measurement .....	page 19
(k) Statistics .....	page 20

PART 3.Consequences of partial/subtotal colectomy, and colon bypass.

(a.i) Diarrhoea after surgery .....	page 21
-------------------------------------	---------

(a.ii)	Faecal output after Right Hemicolectomy .....	page 22
(a.iii)	Faecal output after Left Hemicolectomy .....	page 23
(a.iv)	Faecal output after Subtotal Colectomy or Colon bypass	page 23
(b.i)	Effect of coprophagy on faecal output .....	page 24
(b.ii)	Coprophagy after Subtotal Colectomy .....	page 25
<u>DISCUSSION</u>	.....	page 25

#### FOOD INTAKE.

(c.i)	after Right Hemicolectomy .....	page 33
(c.ii)	after Left Hemicolectomy .....	page 33
(c.iii)	following Subtotal Colectomy .....	page 33
(c.iv)	after Subtotal Colon bypass .....	page 34
<u>DISCUSSION</u>	.....	page 34

#### BODY WEIGHT.

(d.i)	after Right Hemicolectomy .....	page 40
(d.ii)	after Left Hemicolectomy .....	page 41
(d.iii)	following Subtotal Colectomy .....	page 41
(d.iv)	after Colon bypass .....	page 41
(d.v.)	of Subtotal Colectomised rats during a three-weeks period of pair feeding .....	page 42
(e)	Blood picture after Subtotal Colectomy .....	page 42
<u>DISCUSSION</u>	.....	page 42

### PART 4.

#### Changes in Structure and Function.

##### (a) Intestinal Transit-time.

(a.i)	after Right Hemicolectomy .....	page 46
(a.ii)	after Subtotal Colectomy .....	page 47
<u>DISCUSSION</u>	.....	page 48

(b) Intestinal Length and Weight.

(b.i)	The colon after Right Hemicolectomy.....	page 52
(b.ii)	The Colon after Left Hemicolectomy .....	page 55
	<u>DISCUSSION</u> .....	page 56
(b.iii)	The small gut after Right Hemicolectomy .....	page 60
(b.iv)	The small gut after Left Hemicolectomy .....	page 61
(b.v)	The small bowel after Subtotal Colectomy .....	page 61
(b.vi)	The small intestine after Subtotal Colon bypass .....	page 62
(b.vii)	The small intestine in Subtotal Colectomised rats after a three-weeks period of pair feeding .....	page 63
	<u>DISCUSSION</u> .....	page 63

(c) Villus height and enterocyte density.

(c.i)	after Right Hemicolectomy .....	page 76
(c.ii)	following Subtotal Colectomy .....	page 78
	<u>DISCUSSION</u> .....	page 79

PART 5.Barium Sulphate recovery studies.

Introduction and discussion of techniques .....	page 83
Choice of method and marker .....	page 89
Preparation of marked food pellets .....	page 93
Preparation and analysis of samples .....	page 94
Results - barium sulphate recoveries .....	page 95
<u>DISCUSSION</u> .....	page 97

1.

PART 1.

INTRODUCTION AND HISTORICAL REVIEW.

The aim of the work presented in this thesis was to investigate changes in the structure and functions of the rat alimentary tract following removal of part or all of the colon. This was carried out in order to gain information concerning the ways by which the alimentary tract compensates for loss of intestinal functions after partial or total colectomy.

---

In man the main physiological functions of the colon include storage of faeces, to allow one or two rather than several bowel motions per day, and absorption of water and electrolytes. An indirect assessment of the amount of water and electrolytes which the human colon absorbs each day can be made by comparing ileostomy discharge with normal stools (Welch et al., 1936; Phillips, 1969; Turnberg, 1970). Since a well-established ileostomy discharges about 500ml of ejecta per day (Welch et al., 1936; Crawford and Brooke, 1957; Smiddy et al., 1960; Nunguid et al., 1961), it is assumed that the colon in man receives that much of ileal chyme (Hagihara and Griffen, 1972). This chyme consists of 86-94% water (Welch et al., 1936; Kramer et al., 1962); Kanaghinis et al., 1963), as determined by weighing samples of ileal ejecta before and after drying to constant weight, so that the water entering the colon is about 430 - 470ml per day. Analysis of filtrates from well-established ileostomy ejecta indicates that the human colon also receives 40-90m-moles of sodium (Nunguid et al., 1961; Smiddy et al., 1960; Kramer et al., 1962), 20-50m-moles of chloride (Lockwood and Randall, 1949; Fowler et al., 1959; Nunguid et al., 1961) and 2-15m-moles of potassium per day (Crawford and Brooke, 1957; Fowler et al., 1959;

Nunguid et al., 1961; Kanaghinis et al., 1963). By similar procedures it has been estimated that the average amount of water excreted in normal stools is about 150ml per day (Hagihara and Griffen, 1972; Kramer et al., 1962); and that of electrolytes being 2-5m-moles of sodium, 10-20m-moles of potassium and about 2-5m-moles of chloride (Hagihara and Griffen, 1972). On this reasoning the colon in man absorbs about 300ml of water, approximately 70m-moles of sodium and 35m-moles of chloride each day; and secretes up to 4m-moles of potassium per day. These figures are based on the assumption that the ileostomy discharge is the same as the fluid which enters the normal caecum from a normal ileum.

However, results obtained by comparing contents of the terminal ileum in vivo and faeces under standard balance conditions indicate that the amount of water and electrolytes entering the colon in intact healthy subjects is greater than ileostomy discharge measurements would suggest. Thus, by analysing normal faeces, and ileal aspirates sampled for 24 hours from normal subjects, Giller and Phillips (1970) calculated that the colon absorbs 1-1½ litres of water, 137-220m-moles of sodium, 62-123m-moles of chloride and 1-7m-moles of potassium per day. The rectum in man does not absorb water or electrolytes (Devroede and Phillips, 1970), as judged from in vivo perfusion studies in normal subjects.

The colon in animals, particularly herbivorous, in addition to absorbing water and electrolytes, plays an important role in the absorption of energy and synthesis of vitamins. Examination of digesta from all regions of the gastrointestinal tract in sheep, oxen, red deer, horses, pigs, rabbits and rats (Elsden et al., 1946) established that the regions of the alimentary canal in which fermentation and formation



of volatile fatty acids occur are sharply defined and consist of the rumen and reticulum in ruminants and the large intestine in both ruminants and non-ruminants. The amount of volatile fatty acids (VFA) formed in the large bowel of ruminants is small (and probably unimportant nutritionally) in comparison to that in the stomach (Elsden et al., 1946). On the other hand, the large intestine is the only part of the alimentary tract containing significant amounts of VFA in non-ruminants such as the pig, rabbit and the rat (Elsden et al., 1946) and in the fowl (Annison, Hill and Kenworthy, 1968). Significant amounts of VFA are also found in the caeca of guinea-pigs (Hagen and Robinson 1953), porcupines (Johnson and McBee, 1967) and golden hamsters (Hoover, Manning and Sherin, 1968); and represent the major fraction (50-60%) of the titratable organic anions in normal human stools (Rubinstein et al., 1969). These fatty acids have been shown by chromatographic analysis of digesta distillates to consist mainly of acetic, propionic and butyric acids (Elsden et al., 1946; Yang, Manoharan and Mickelson, 1970; Hernning and Hird, 1972).

The proof of VFA absorption from the large intestine rests on the fact that significant concentrations of acetic, propionic and butyric acids are found in the blood draining the caecum and colon, e.g. of the horse, pig and rabbit (Bancroft et al., 1944) and the porcupine (Johnson and McBEE, 1967). Analysis of venous blood samples from the caecum or the colon, and of caecal or colonic contents taken at the same time as the blood samples show that the VFA present in the caecal and colonic contents are of the same kind as those present in the blood samples (Bancroft et al., 1944).

In the rat, recovery of radioactive carbon dioxide in the expired air together with measurement of rates of disappearance of radioactivity

from the caecum and the colon of rats killed at various intervals after injection of labelled VFA into the caecum (Yang et al., 1970) confirm that these acids are absorbed from the large intestine and metabolised. By feeding rats a test meal containing labelled acetic, propionic and butyric acids and measuring radioactivity in liver glycogen isolated from these animals (Buchanan et al., 1943), it has been demonstrated that VFA are converted into glycogen. Thus, the fate of VFA in ruminants and in non-ruminants is the same i.e. these acids are metabolised to provide energy, or converted into glycogen and lipids (Lorber et al., 1959; Ballard et al., 1969) and stored for later use.

The nutritional contribution from volatile fatty acids in non-ruminants whose natural diets contain significant amounts of cellulose may be considerable. In the rat, for example, the energy contributed by caecal VFA, as calculated from the amount of VFA disappearing from the caecum of rats fed a normal chow and killed at intervals after the last meal (Yang, Manoharan and Mickelson, 1970) is estimated at 4.7% of the daily energy intake. In the wild porcupine fermentation rates determined by the zero-time method, using caecal contents from slaughtered animals, indicated that from 16 to 33% of the energy requirement is supplied from caecal VFA (Johnson and McBee, 1967).

There is evidence that important synthesis of vitamin B complex and of vitamin K occurs in the colon of such animals as pigs (Nasr, 1950; Barber et al., 1953), rats (Michelson, 1956; McGregor et al., 1947; Johnsson et al., 1953), rabbits (Kulwich, Strugila and Pearson, 1953; Huang et al., 1954), chicks (Couch et al., 1950) and chickens (Simonnet et al., 1953); so that colectomised animals might fail to grow and thrive through deficiency of these vitamins.

The colon both in man and animals also absorbs bile acids.

Measurement of absorption of  $^{14}\text{C}$ -labelled cholic acid both by fall in luminal bile concentration and by isotope excretion in fistular bile following injection of the labelled bile acid into the lumen of either the caecum or the colon (Samuel et al., 1968) suggest that the colon in man is capable of absorbing about 60% of the bile acids reaching it. However, since less than 10% of the bile acids entering the small intestine reaches the colon (Weiner and Lack, 1968), the rest being absorbed mainly from the distal ileum (Borgström, Ludh and Hofmann, 1963), the colon in intact man probably does not play an important role in maintaining the size of the bile acid pool or the intraluminal concentration of bile acids necessary for normal fat digestion and absorption (Kim et al., 1968). Whether bile acid absorption from the human colon can be increased to partially compensate for loss of active bile acid transport resulting from resection of the ileum is not known (Perry, Mok and Dowling, 1974). In vivo perfusion studies using radioactive bile acids in the rat, an animal in which measurements of  $^{14}\text{C}$ -labelled bile acid transport have confirmed absorption of bile acids from the large intestine (Norman and Sjöval, 1958; Holt, 1964; Sullivan, 1965) amounting to about 70% of the bile acids reaching the colon (Lindstedt and Samuelson, 1959), have shown that the colon, at least in this animal, does not adapt to the absence of the ileum by increasing its absorptive capacity for bile acids (Perry et al., 1974).

In view of the important functions performed by the large intestine in man and animals, it seems strange that colectomy does not cause important complications. In man, although the ability to survive on very low salt intake (less than 5m-moles of sodium chloride per day) for long periods without developing symptoms and signs of sodium depletion (Dole et al., 1950) seems to be severely impaired in subjects

with long-established ileostomy (Gallagher et al., 1962; Kramer, 1966), and there may be a tendency for healthy subjects with well-established ileostomy to become more easily depleted of water and electrolytes than intact healthy subjects when exposed to episodes of mild gastroenteritis, or excessive sweating during hot spells (Gallagher et al., 1962), removal or bypass of the colon does not significantly disturb bowel functions. Immediately following removal or bypass of the colon, with formation of either an ileostomy or ileoproctostomy, watery stools tend to occur 8-15 times a day (Best, 1948; Best, 1952), and the loss of water and electrolytes in the faeces is great enough to require replacement therapy (Lockwood and Randall, 1949; Crawford and Brooke, 1957; Kanaghinis et al., 1963); but with recovery the stools gradually change from fluid to semi-solid, and the frequency of bowel motion eventually reduces to 2-3 evacuations per day (Ravitch, 1948; Lillehei and Wangenstein, 1955; Teicher and Abrahams, 1956; Webster and Howard, 1973; Coleman and Eckert, 1955); the loss of water and electrolytes in the faeces drops, although it remains far in excess of that in normal faeces even when the ileostomy has been established for years (Lockwood and Randall, 1949; Phillips, 1969; Turnberg, 1970). The same trend of events takes place also in the dog (Ravitch, 1947) and the rat (Lambert, 1965; Wright et al., 1969b): after removal of the large intestine the stools change from liquid to soft as time passes.

The mechanism of adaptation of intestinal absorption after colectomy has not been established, and the changes in the alimentary tract which may lead to restoration of bowel functions after colectomy are still incompletely known. Histological, and in vivo water and sodium absorption studies in man (Wright et al., 1969a) and the rat (Wright et al., 1969b) provided results which suggest that the ileum may

take over the absorptive function of the colon: both the ileal absorptive surface (as judged from villus height and the number of mucosal epithelial cells from crypts to the tips of villi) and the absorption of water and sodium were increased, in man and in the rat. However, these studies were limited to the terminal ileum; and there is lack of information in literature concerning adaptive changes (if any) in the rest of the small intestine; whether or not morphological and functional adaptive changes similar to those observed in the terminal ileum develop along the entire length of the small gut after colectomy remains to be investigated. Also, whether the restoration of bowel functions after partial colectomy is brought about by structural and/or functional changes in the small intestine, or the remnant of colon, is not known.

In this work, therefore, experiments were carried out to determine the adaptive changes in the structure and some functions of the alimentary tract following removal of part or all of the large intestine. It was thought appropriate that these aspects should be explored in the rat because of a liberal supply, uniform size, cheap maintenance and the animal being omnivorous like man.

---

## PART 2.

### PLAN OF WORK.

The work aimed to carry out colectomy, and colon bypass, in rats and to assess the consequences as fully as possible. The measurements included: 1) body weight, 2) food intake, 3) faecal output, 4) energy absorption, 5) intestinal transit-time, 6) blood picture, 7) gut weights 8) intestinal length, 9) villus height, and 10) enterocyte density.

---

### Techniques.

#### (a.i) Animal Care.

All experiments reported in this thesis were performed on male albino rats of the Wistar strain. They were received at two months of age, from the University centre for laboratory animals, the Bush, Midlothian, where they had been bred as Specific-Pathogen-Free (SPF) animals. They were free from all helminthic and protozoan infections, pasteurellosis and pneumococcal pneumonia, chronic respiratory diseases caused by streptobacillus moniliformis, mycoplasma pulmonis, bacillus muris and bordetella bronchiseptica; and intestinal infections caused by salmonella and shigella. Some rats were obtained, at two months of age, from litters locally bred in the animal house (a minimum disease unit) of the department of Physiology, University of Edinburgh Medical School. The SPF rats were allowed at least two weeks to settle down in the animal house of the department of Physiology before they were taken for experiments; during this period of adapting to a new environment, they ate and gained weight steadily.

All animals were housed under conditions of controlled temperature

Table 1.

Rat Diet Prepared by McGregor and Co. (Leith) Ltd. 1974.

<u>Food Matter</u>	<u>Content %</u>
Ground Wheat	21.87 %
Ground Oats	17.50 %
Maize Meal	20.00 %
Barley Meal	7.47 %
Meat & Bone Meal	8.74 %
Fish Meal	5.00 %
Dried Skimmed Milk	7.47 %
Whey Powder	6.27 %
Unextracted Dried Yeast	1.27 %
Molasses	2.47 %

---

	<u>Amount per Kg</u>
Salt (NaCl)	42 (g)
Vit. A (in gelatin base)	12,000 units
Vit. D	3,000 units
Vit. E	2.4 mg
Vit. K (Menadione bi-sulphate)	1.5 mg
Calcium Phosphate	0.2 mg

---

Moisture content	10.5 %
Protein	21.2 %
Carbohydrate	64.8 %
Fat	4.1 %
Fibre content	3.4 %

(70°F) and day length (12 hours light and 12 hours darkness), with free access to water and a commercial normal diet for rodents; diet GR3.EK, supplied by McGregor and Co. (Leith) Ltd. This was supplied in a pellet form, and consisted of approximately 64% carbohydrates, 21% protein, 4% fat and 10% water; and was fortified with vitamins A, D and K (for detail on composition of diet see table 1).

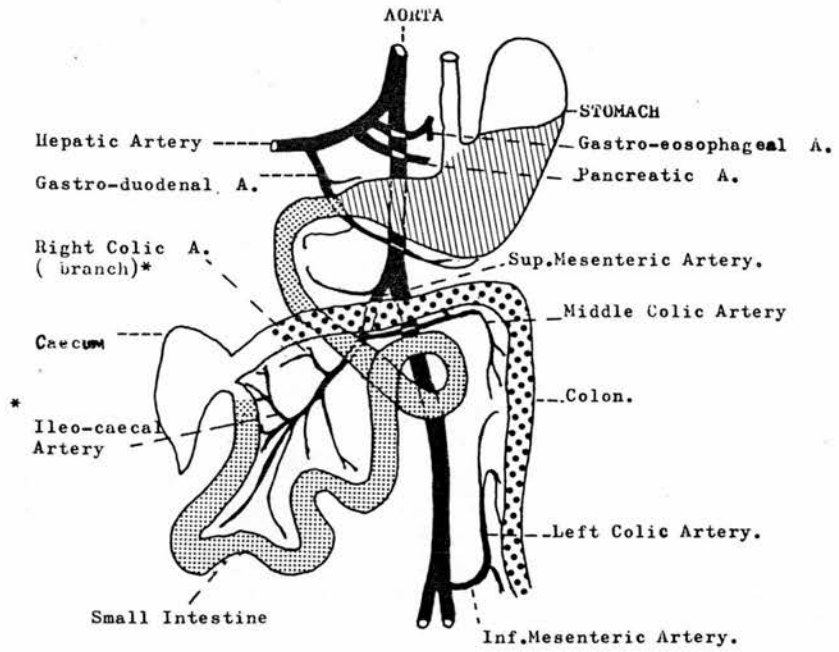
Since all animals used in this work had previously been housed in groups, it was thought necessary to keep them at least in pairs, so as to avoid abnormal physiological responses caused by separation. Isolated rats are known to grow less well than those kept together (Donaldson, 1924); presumably because of reduced food intake in the solitary rats (Harlow, 1932; McDonald et al., 1963). On admission all rats were weighed and paired according to body weight. Each pair was kept on racks in a wire-bottom polystyrene cage, 38cm X 25cm X 18cm, with a tray underneath to serve as a receptacle for stools and scattered food.

(a.ii) Allocation of animals to various experimental groups.

Pairs were allocated together as pairs by toss of coin to one of two major categories: pairs for operation, or pairs for no operation. The operations comprised: right hemicolectomy, left hemicolectomy, subtotal colectomy, subtotal colon bypass. At any operating session, only one of these operations was done, usually on 4 rats, and these rats were chosen from 4 pairs for operation as the first animals picked from each cage at one session, or the second animal at the next session, and so on. The other rat of each pair was subjected to sham operation (ileal transection and anastomosis).

Throughout the post-operative period animals were kept in pairs according to the type of operation; so that the preoperative pairs were separated. However, the partners in each old pair were still





**Fig. 1a.**

A diagrammatic presentation of the major arterial blood supply to the gastrointestinal tract of the rat.  
 \* Vessel ligated when the proximal colon (unshaded portion) was removed.

identifiable by means of tail markings (with permanent Indian ink); and those which survived the experiments provided data for Student's paired 't' test.

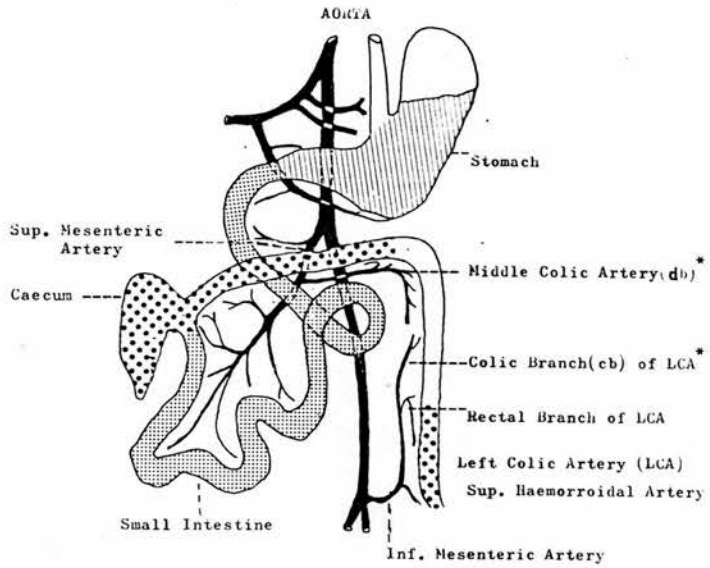
In some of the experiments both unoperated and sham operated rats were used as controls. To ascertain whether or not metabolic changes resulting from surgical trauma (Cuthbertson, 1959) contribute to changes in the gut structure, sham operated controls were used. However, since simple intestinal transection has been observed to induce the rat's small intestine to develop hypertrophy (Loran and Althausen, 1958) it was thought necessary also to include unoperated animals as controls.

#### (b) Operative Procedures.

Animals were left without food for 24 hours before and after operation, but were allowed water. Surgical anaesthesia was induced by an intraperitoneal injection of pentobarbitone (veterinary Nembutal) at a dose of approximately 3mg/100g of body weight. The skin of the abdominal wall, from just below the xiphoid cartilage to the root of the penis, was clean shaven.

##### (b.i) Right Hemicolectomy (Caecectomy).

Following a midline incision, the caecum was identified and brought out of the abdomen. It was placed on a moist swab on the abdominal wall. The terminal loop of ileum was also exteriorised. The ileocaecal artery (see Fig. 1a) lying in the root of the mesentery between the terminal loop of ileum and the colon was found and ligated with a No. 6/0 silk suture. Division of the small intestine and of the proximal colon was carried out as described by Lambert (1965). The whole of the intestine supplied by the colic branch of the ileocaecal artery, i.e. about 5mm of terminal ileum, the whole of caecum and about



**Fig. 1b.**

A diagrammatic presentation of the arterial blood supply to the distal colon in the rat.

\* Vessel ligated when distal colon (unshaded portion) was removed.

15mm of the colon immediately after the caecum was removed. It was found necessary to tie the branch of the right colic artery which anastomoses with the ileocaecal artery (Fig. 1.a.). Continuity of the gut was re-established by an end-to-end anastomosis of the ileum to the colon by means of interrupted, invaginating single layer stitches as described by Lambert (1965).

(b.ii) Left Hemicolectomy (Distal colon resection).

Surgical anaesthesia was induced, and the abdominal cavity entered by the same procedure as described for right hemicolectomy. The distal colon was exposed by tucking the overlying loops of intestine under the left abdominal wall. Next, the middle colic artery was identified, and the descending branch (indicated 'db' in Fig. 1.b.), which anastomoses with the left colic artery was ligated. Also, the colic branch (indicated 'cb' in Fig. 1.b.) of the left colic artery was tied just above the origin of the branch supplying the rectum. Resection of the segment of colon supplied by both the descending branch of the middle colic artery and the colic branch of the left colic artery was carried out. About 3 to 5cm of colon was removed. To re-establish continuity of the gut, the rectum was anastomosed, end-to-end to the remaining colon, using a No. 6/0 silk suture for interrupted, invaginating single layer stitches.

(b.iii) Subtotal Colectomy.

The caecum and terminal loop of ileum were exteriorised, the ileocaecal artery tied, and the ileum sectioned at about the same place as described in (b.i.). The right colic and middle colic arteries were identified and ligated. The colic branch of the left colic artery was also tied; and resection of the entire large intestine,

from the caecum to about the same level as the origin of the rectal branch of the left colic artery was carried out as described by Lambert (1965). Continuity of the gut was re-established by an end-to-end anastomosis of the ileum to the colon stump by the same method as in (b.i.) and (b.ii.).

(b.iv.) Subtotal Colon Bypass.

After the caecum and the terminal loop of ileum were brought out of the abdominal cavity, the ileum was sectioned as described for right hemicolectomy. But instead of tying the ileocaecal artery, only the segment of vessel connecting the colic and ileal branches of this artery was ligated. The opening of the ileal stump on the caecum was closed with a purse-string suture using a No. 6/0 silk; and an end-to-side anastomosis made between the free end of the ileum and the distal colon at about the same place as the anastomosis in (b.ii.). Single layer interrupted, invaginating stitches were employed.

(b.v.) Ileal Transection and Anastomosis.

In sham operated animals, the ileum was exteriorised by the same procedure as described for right hemicolectomy. Transection and anastomosis was done at the point where the part supplied by the ileal branch of the ileocaecal artery meets the colic branch of the same artery, i.e. about the same level as the ileal section in (b.i.).

(c) Food Weighings.

The daily food consumption for each pair of rats was measured by weighing food containers together with food pellets at the beginning and end of 24-hour feeding periods. The weight recorded after feeds

was subtracted from the weight before feeds; the difference gave the amount of food eaten and scattered. From this the actual food intake was obtained by deducting the weight of scattered food. But since the spilled food was usually damp with urine at the time of collection, it had to be dried to constant weight before deductions were made. The weights of eaten and scattered food were also converted to dry weights by correcting for water content in the diet (which averaged 8.42g/100g of food, as judged from 10 samples dried to constant weights in a hot air oven at 85°C constant temperature). Food intake, therefore, was measured in grams of dry food matter per pair of rats per 24 hours; and the approximate amount consumed by each rat was obtained by dividing the 24-hour food intake of the pair by two.

A Berkel Auto-Scale balance, accurate to 0.1g, was used to weigh food-hoppers together with pellets; and an Ultramatic balance, model U.M.3 (Stanton Instruments Ltd.) accurate to 0.1mg was used for the scattered food and the dried samples.

#### (d) Faecal Weighings.

Stools were collected after every 24 hours, and dried to constant weights as described for food samples. Because of diarrhoea in the early post operative period, it was thought likely that colectomised rats would not eat their faeces, a situation likely to cause increased faecal output (Barnes, Kwong and Fiala, 1958). For this reason, the significance of coprophagy on the amount of faeces passed per day was tested in 24 normal rats. Stools were collected by grab sampling technique at 2-hourly intervals for 24 hours. Rats were coaxed to defecate directly into specimen bottles held over the anus. When they failed to pass stools spontaneously, faeces were manually removed from

the ano-rectal canal by applying gentle digital pressure over the loaded canal.

In one series of experiments the content of water in the stools was determined by weighing grabbed samples immediately after collection and after drying to constant weight in a hot air oven at 85°C. The amount of water present in the faeces, indicated by the difference between wet and dry weights, was expressed as a percentage of the wet weight.

To check whether stools collected at different times of day contained the same amount of water, 2-hourly grab samples were collected from 24 normal rats, during a 24-hour period, and analysed for water content.

#### (e) Intestinal Transit-time.

Transit of barium sulphate suspension (Micropaque) through the small intestine was studied after 3ml of barium sulphate was administered intragastrically in rats fasted overnight (12 hours). The test meal was injected through a stomach tube, as described by Machella and Griffith (1949). Each animal was then placed in a plastic box, approximately 28cm X 12cm X 9cm, fitted with a transparent perforated lid. The gastro-intestinal tract was radiologically examined by screening rats at 25 - 30 minute intervals until the small intestine was completely clear of barium. The progress of the meal was studied under an image intensifier during 60 to 90 seconds exposure periods.

The stomach-to-anus transit-time was studied in subtotal colectomised and unoperated rats following an intragastric injection of barium sulphate by the above technique. After giving the barium in the evening, faeces were collected from trays at frequent intervals

and dried in a hot air oven at  $85^{\circ}\text{C}$  for 24 hours before they were examined by radiography.

(f) Energy Absorption.

Measurement of dietary energy absorption by whole animal balance technique (method described and discussed in detail in part 5 of this thesis) was carried out in subtotal colectomised and sham operated rats three months after surgery. Animals were housed individually for one week before, and during, the study. Metabolism cages with the diet compartment removed from the main cage were not available, so that spillage of food into the faeces could not be avoided. Furthermore, no facilities for diverting urine from faecal droppings and scattered food were available. To avoid inexact separation of damp food from damp stools, particularly so when the faeces passed are loose (as in subtotal colectomy), it was necessary to dry the faeces before separation could be undertaken. The 24-hour pooled faecal droppings and scattered food were first allowed to dry at room temperature ( $70^{\circ}\text{F}$ ) for 24 hours. After separation and complete collection the stools and scattered food were dried to constant weights in a hot air oven at  $85^{\circ}\text{C}$ . Food intake was calculated as described in (c).

The daily faecal dry matter (measured for 6 operated and 6 sham operated rats) from each animal was powdered and aliquots taken for bomb calorimetry; these were stored at  $4^{\circ}\text{C}$  until analysed.

(g) Blood.

Tail blood samples were taken at 2, 4 and 12 weeks after surgery from rats with subtotal colectomy and from sham operated controls. The following were determined: 1) haemoglobin concentration, 2) total white



blood cell count, 3) red blood cell count, 4) mean corpuscular volume, 5) mean corpuscular haemoglobin, and 6) mean corpuscular haemoglobin concentration, Blood samples were collected with capillary pipettes into ethylenediamine-tetra-acetate (EDTA) solution prepared 18-24 hours previously, and were analysed within 30 minutes after collection, using an automated Coulter Electronic (Model S) counter.

#### (h) Intestinal Weight and Length Measurements.

Operated and control animals were killed with chloroform 2 to 4 months after surgery. Preliminary fasting was not observed because there was no way of telling whether loss in mucosal mass due to fasting (McManus and Isselbacher, 1970) would be of the same magnitude in operated and in control animals. All animals were killed between 1300 and 1600 hours.

As soon as the animal died, the entire intestinal tract, i.e. from the pyloroduodenal junction to the level of the pubic symphysis (which happens to be approximately the level at which the colon was anastomosed to the terminal ileum in subtotal colectomy and colon bypass, or to the rectum in distal hemicolectomy), was removed. The small intestine was detached from the large bowel by a transection at either the ileocaecal junction or the ileocolic anastomosis. Both intestines were cleaned of mesentery and fat, and the bowel contents washed out with a 156m-molar Sodium chloride solution from a syringe. The caecum and colon were slit open to allow easy washing and examination for macroscopic inflammation or worm infestation. An inflamed or worm infested intestine was discarded.

#### (h.i) Length Measurement.

Measurements of length were made on the fresh small intestine, or

the colon (excluding the caecum). The intestine was suspended against a vertical scale (150 cm high), and a weight, 6.8g, attached to the lower end of the suspended gut to keep it stretched down. In studies which required the small intestine to be divided into segments of equal length, the intestine was divided as it hung against the scale. First, the gut wall was incised at equally spaced sites along the length, to mark off the segments. Transection at these sites divided the intestine into portions of equal length.

(h.ii) Weight Measurement.

Wet and dry weights of the small or large intestine were measured for mucosa and seromuscular coat together (whole gut weight), and for seromuscular coat and mucosa separately. Specimens were first dabbed between tissue paper, to remove excess of absorbed saline, before wet weights were measured. Filter paper was not used because it tended to soak up not only saline solution, but mucosa as well. Dry weights were obtained from specimens dried to constant weight as described for stool and food samples. After 4 days of drying all specimens attained constant weights. The same Ultramatic balance used for weighing dried stools and food samples was used also for weighing gut weights.

It must be noted here that the only weight obtained from the undivided small intestine was wet weight. Usually the small bowel was divided into three segments of equal length by the method already described. In this work these segments are referred to as the upper, middle and lower thirds of the small intestine.

(h.iii) Mucosal separation by scraping (Gleeson, Dowling and Peters, 1972) was carried out on all the three segments of small bowel taken from operated and control rats. A pilot study involving examination of

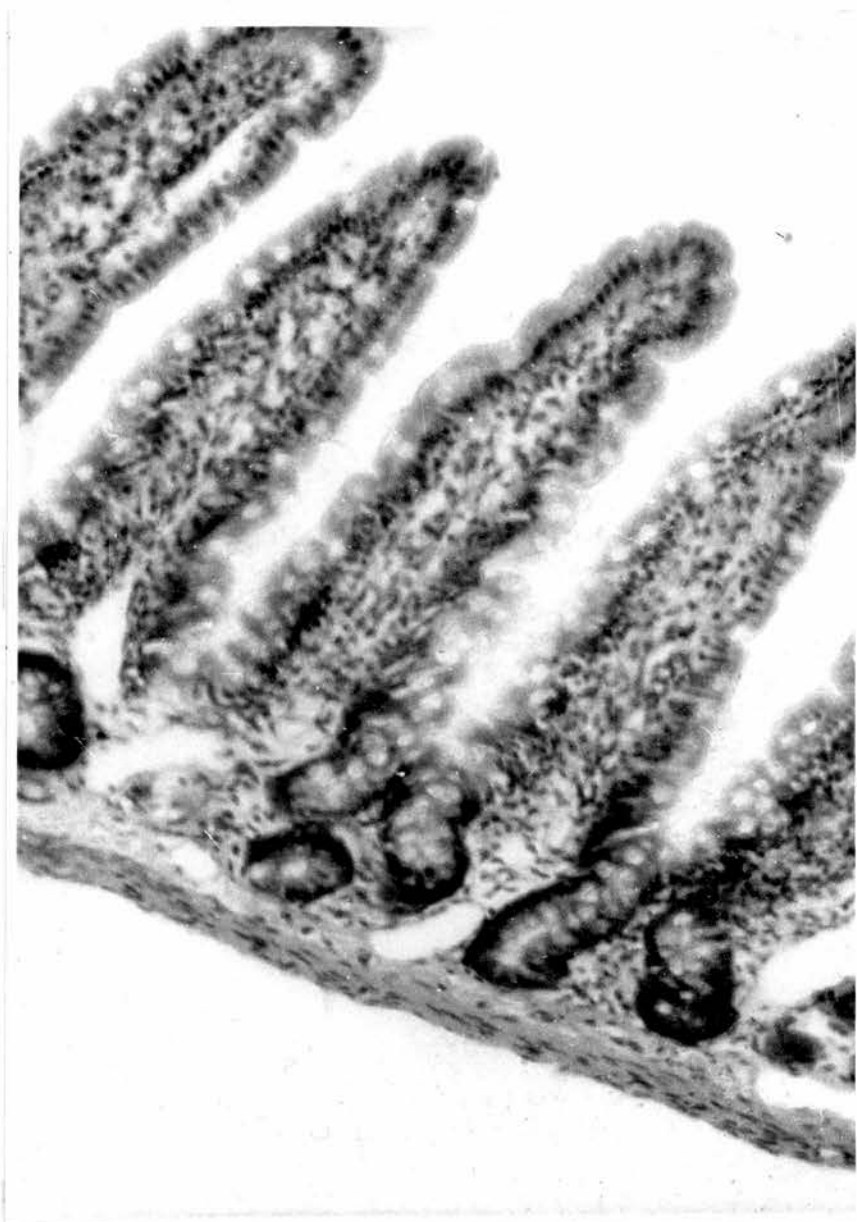


Fig. 2a.

Section through normal small gut wall showing mucosa and seromuscular coat together.

microtome sections of the small intestinal wall showed that mucosa was completely removed from the seromuscular coat by scraping (Figs. 2a and 2b). In one group of rats mucosal separation was also carried out on the colon (excluding the caecum); separation was complete (Figs. 3a and 3b). Whenever mucosa was separated from the seromuscular coat, dry weight for the whole intestine (undivided) was obtained by adding up the weights of mucosa and seromuscular coat from the upper, middle and lower segments.

#### (j) Villus Height and Enterocyte Density.

##### (j.i) Preparation of Histological sections.

These studies were carried out 3 to 4 months after operation, and for obvious reasons were confined to the small intestine. To minimize autolysis, fixation of the small bowel was started in vivo. Animals were anaesthetized with either ether or intraperitoneal pentobarbitone, and the abdominal cavity entered through a midline incision. The small bowel was detached from the large intestine at either the ileocaecal junction or the ileocolic anastomosis. The terminal loop of ileum was brought out of the abdomen so that bowel contents could be washed out without flooding the abdominal cavity. A cannula was inserted into the duodenum, and 10% aqueous formalin allowed to gravitate into the gut from a 250ml aspirator through a connecting rubber tube. After the bowel contents were washed out, the small intestine was mildly distended with formalin and left in situ until the animal died. Animals usually survived and maintained good circulation (as judged from pulsation of mesenteric arteries) for at least 5 minutes after distension of the bowel.

As soon as the animal died the small intestine (also the large bowel)

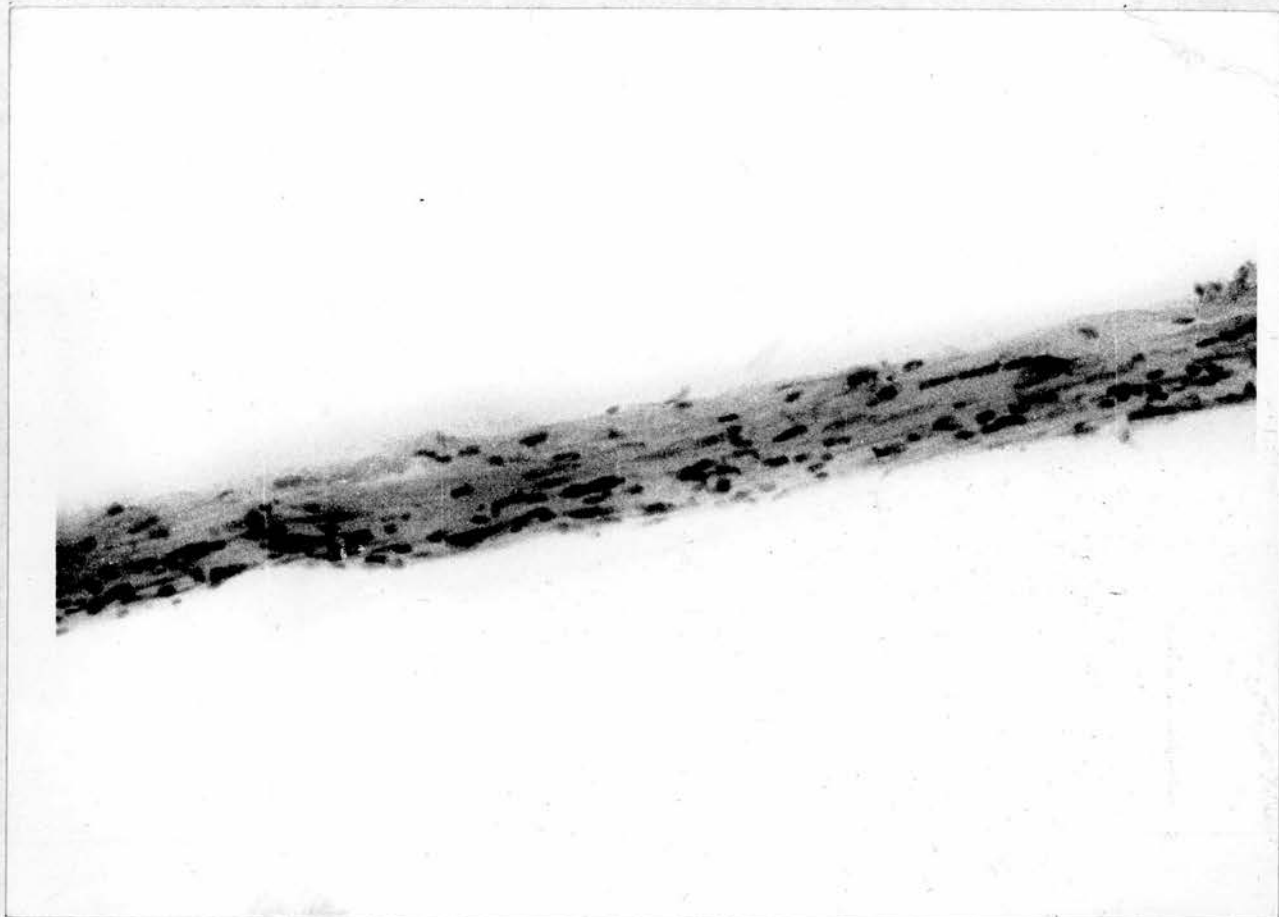


Fig. 2b.

The seromuscular coat of the small gut after complete mucosal separation by scraping.

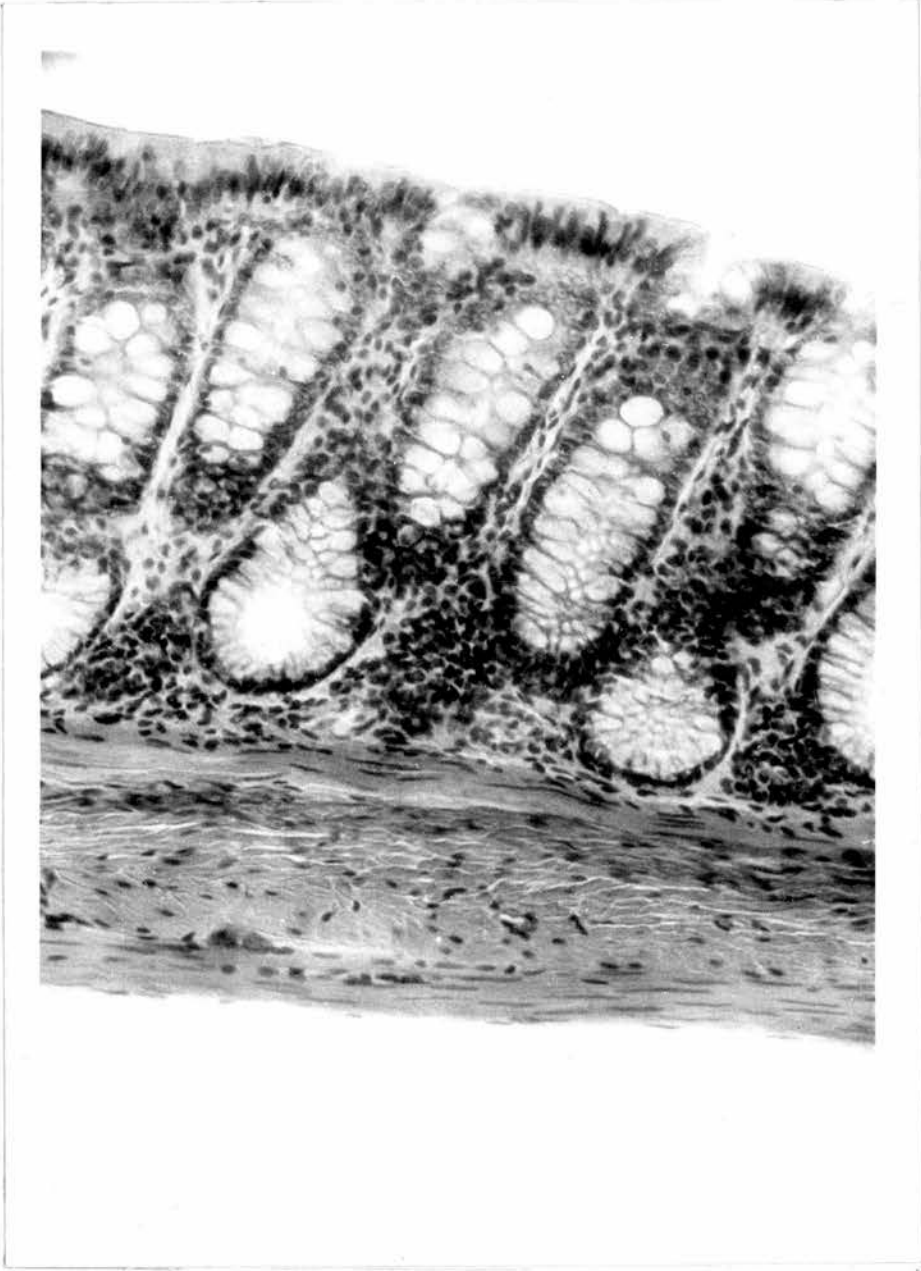


Fig. 3a

Section through normal colon wall, showing mucosa and seromuscular coat together.

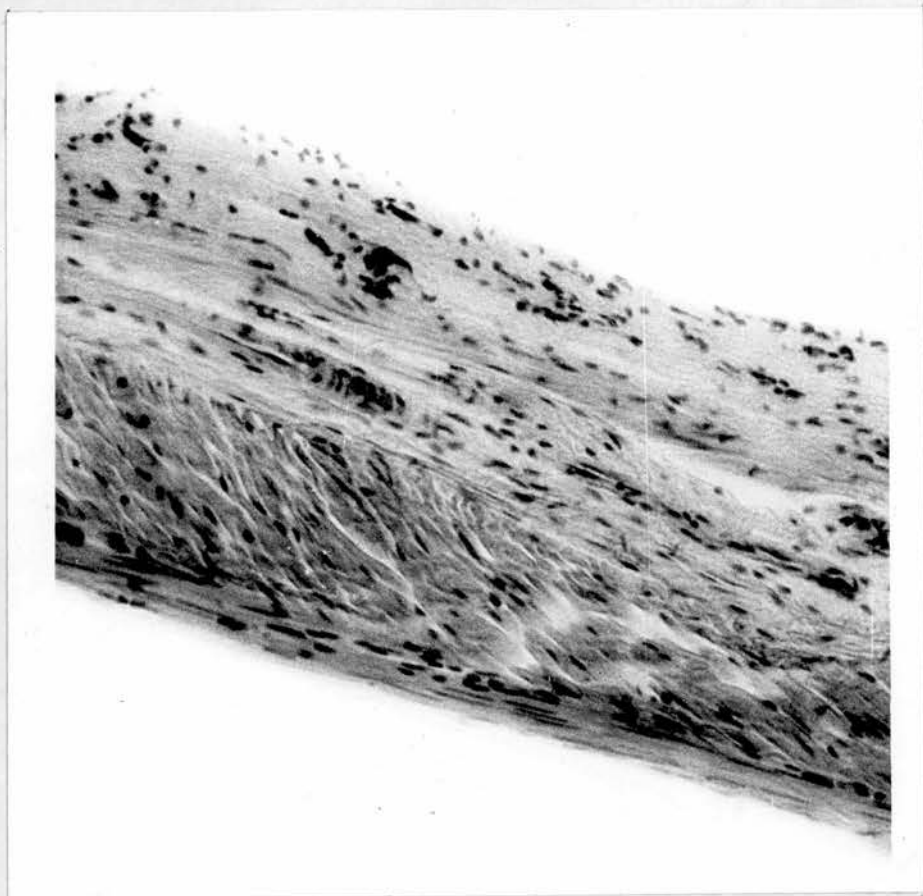


Fig. 3b.

The seromuscular coat of the colon after complete mucosal separation by scraping.

was removed and cleaned of mesentery and fat. During this time the gut still contained formalin. It was then submerged in 10% aqueous formalin in a trough (150cm long) and stretched out to be fixed for 24 hours under an intraluminal pressure of about 10cm of formalin (as judged from the level of formalin in the aspirator held 5cm above the gut). Without these precautions it was found that preparations usually showed much damage.

Length measurement of the intestine, by the method described before, was taken after the 24 hours fixation, and the gut divided into 30 portions of approximately equal length. These represent 30 levels, from duodenum to terminal ileum, at which microtome sections of the gut wall were taken. Routine dehydration, clearing and paraffin embedding followed. Microtome sections, 10 micrometers thick, were cut transverse to the long axis of the gut. One slide, with at least 10 serial sections taken from midsegment, was made for each of the 30 segments and was stained with haematoxylin -eosin.

(j.ii) Measurement of Villus Height and Enterocyte Density.

A calibrated graticule was used to measure villus height and enterocyte density, i.e. the number of enterocytes per unit length of villus edge, rather than per unit area of villus surface. From each slide 30 villi were measured; only the 5 well oriented tallest villi from each of 6 sections on the slide were taken. Epithelial cell counts were taken from all villi measured for length. Counting was confined to 150  $\mu\text{m}$  of mid villus edge, and included all nuclei of epithelial cells. The nuclei of migrating lymphoid cells could be easily recognized and were not counted. In sections where the villi were short, and the midvillus edge less than 150  $\mu\text{m}$ , counts were taken from two villi to cover the required length.



To avoid bias, the identity of the slide (numbered by scratching with a diamond marker) was not looked for until counting was completed: the number on the slide was covered with a mounting material, DPX, at the time slides were mounted. After counts and villus height measurements had been taken the number was exposed by scraping off the DPX.

(j.iii) Calculation of Magnification Factor.

A circular graticule of diameter 3.050mm was placed on the stage of a light microscope used in this work. The calibrated graticule used for measuring villus height and enterocyte density was mounted in the eye piece lens-holder. Under 10 X 10 magnification (used for measuring villus height) the 3.05mm was covered by 227 divisions of the calibrated graticule. By simple division:  $3.05\text{mm} \div 227 = 0.013\text{mm} = 13 \mu\text{m}$ . The villus height was obtained by multiplying the number of graticule divisions measured for the villus by  $13\mu\text{m}$ . No correction was made for shrinkage.

Similarly, the value, in length, for each division on the calibrated graticule was calculated under the magnification of 10 X 40, used for enterocyte counting. Under this magnification each division represented  $3\mu\text{m}$ ; 50 divisions were required to cover  $150\mu\text{m}$  of midvillus edge.

(k) Statistics.

Unless stated otherwise statistical significance of results presented in this thesis were computed from a Student's paired 't' test, with Bessel's correction. Throughout the thesis the value after  $\pm$  sign is the "s.e.m."

PART 3.RESULTS.(a) Condition of Animals After Operation.(a.i) Diarrhoea.

During the first two to three weeks after operation, all rats with right hemicolectomy developed diarrhoea (passing semi-fluid stools), but otherwise remained in good health. After the third post-operative week, diarrhoea was replaced by ill-formed pellets, which continued to be passed throughout the duration of the study. Mortality rate was about 10%; death usually occurred within the first three days after surgery, and was caused, apparently, by small bowel obstruction due to paralysis. Death occurring weeks or months after the operation resulted from intestinal obstruction caused by adhesions.

In contrast, diarrhoea did not occur after distal colon resection: in all operated animals the first postoperative stools were in a pellet form; and although faeces passed during the first week after operation were relatively soft when compared to normal rat stools, after this period all animals with left hemicolectomy continued to pass faeces which were of the same consistency as those passed by normal rats. No animal in this group died.

On the other hand, rats subjected to either subtotal colectomy or colon bypass developed very severe watery diarrhoea during the first three to four weeks after operation. In some animals the passage of loose stools was very frequent, and caused excoriation of the perianal and scrotal skin. However, this diarrhoea gradually subsided; and after the sixth postoperative week nearly all animals with subtotal colectomy or colon bypass were passing semi-solid stools, which remained

unformed throughout the study period. Also, the skin excoriation gradually disappeared. In spite of the severe<sup>e</sup> diarrhoea in these animals, the mortality rate was the same as in the right hemicolectomy group; and the apparent cause of death was the same.

Sham operated rats had an uneventful postoperative life, although 2 out of 50 animals died of small bowel obstruction resulting from adhesions, two and three months after operation.

(a.ii) Faecal Output After Right Hemicolectomy.

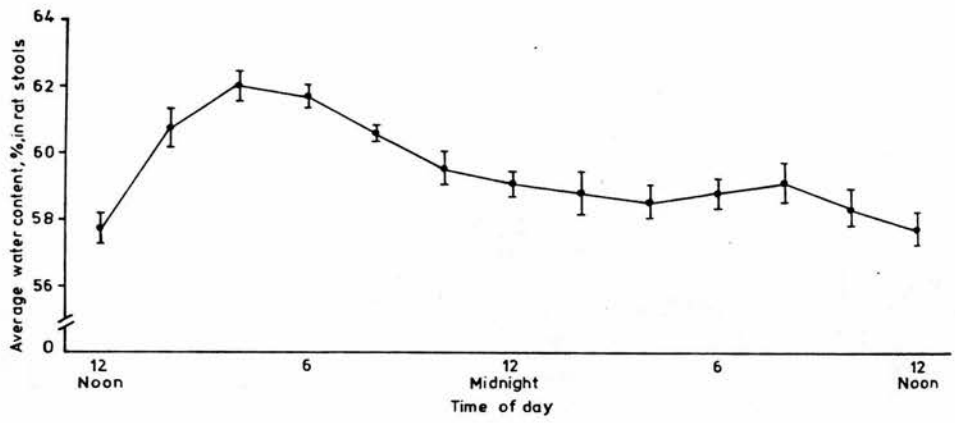
Results obtained from 24-hour faecal collections carried out during the second week after operation, daily for 7 days, from 8 operated, 8 sham operated and 8 unoperated rats indicated that

they excreted on average  $6.7g \pm 0.3$ ,  $4.9g \pm 0.1$  and  $5.2g \pm 0.3$  per rat, respectively. The output in right hemicolectomised rats was significantly greater than in either sham operated or unoperated controls,  $p < 0.001$ .

Measurements taken during the eighth postoperative week, for three days, revealed similar significant differences between the daily faecal dry matter output in operated and control animals. Indeed, the operated rats were passing about the same amount as during the second week.

The water content in stools collected between 0930 and 1030 hours from rats with right hemicolectomy, two weeks after operation, was about 20% above control values: faeces passed by operated rats were 69.6% to 98.1% water, the mean value being  $75.8 \pm 1.1\%$ . During the same period, sham operated controls passed stools consisting of 50.6% to 61.9% water, the average being  $56.1 \pm 0.1\%$ . The difference is highly significant,  $p < 0.001$ .

Eight weeks after operation the average water content in the faeces



**Fig. 4.** Water content (mean  $\pm$  s.e.m.) in normal rat stools collected over 24 hours.

of right hemicolectomised rats (collected between 0930 and 1030 hours) dropped to  $69.1 \pm 1.2\%$ . When compared to that in the control stools, i.e.  $57.1 \pm 0.8\%$ , the difference is still highly significant,  $p < 0.01$  though much smaller; when stools passed by operated rats during the second postoperative week are compared to those passed eight weeks after operation, the difference in water content (i.e. between  $75.8\%$  and  $69.1\%$ ) is significant,  $p < 0.01$ .

In presenting results on faecal water content reference has been made to the time samples were collected. This is because the water content in grab samples, collected from 24 normal rats over 24 hours, varied with time and displayed a diurnal rhythm (Fig. 4). Stools passed at 1200 hours had the lowest water content,  $55.4$  to  $60.1\%$ , and a mean of  $57.9 \pm 0.1\%$ . The highest values were observed at 1600 hours, when the water content reached an average of  $62.1 \pm 0.4\%$ , with a range of  $60.5\%$  to  $63.8\%$ . The difference between the highest and lowest observed values is highly significant,  $p < 0.01$  (computed 't' test).

(a.ii) Faecal Output After Left Hemicolectomy.

Left hemicolectomy had no significant effect on faecal output. Thus, during the third week after operation the average faecal dry matter output per rat, measured over 6 days for 8 left hemicolectomy and 8 control animals, was  $4.8g \pm 0.1$ ; and  $4.5g \pm 0.2$ , respectively. Water content was not measured.

(a.iii) Faecal Output After Subtotal Colectomy.

The daily faecal dry matter output after subtotal colectomy increased by 50 to 60%. During the second postoperative week, 24-hour faecal collections carried out for three days, from 8 operated, 8 control operated and 8 unoperated rats gave an average output per rat

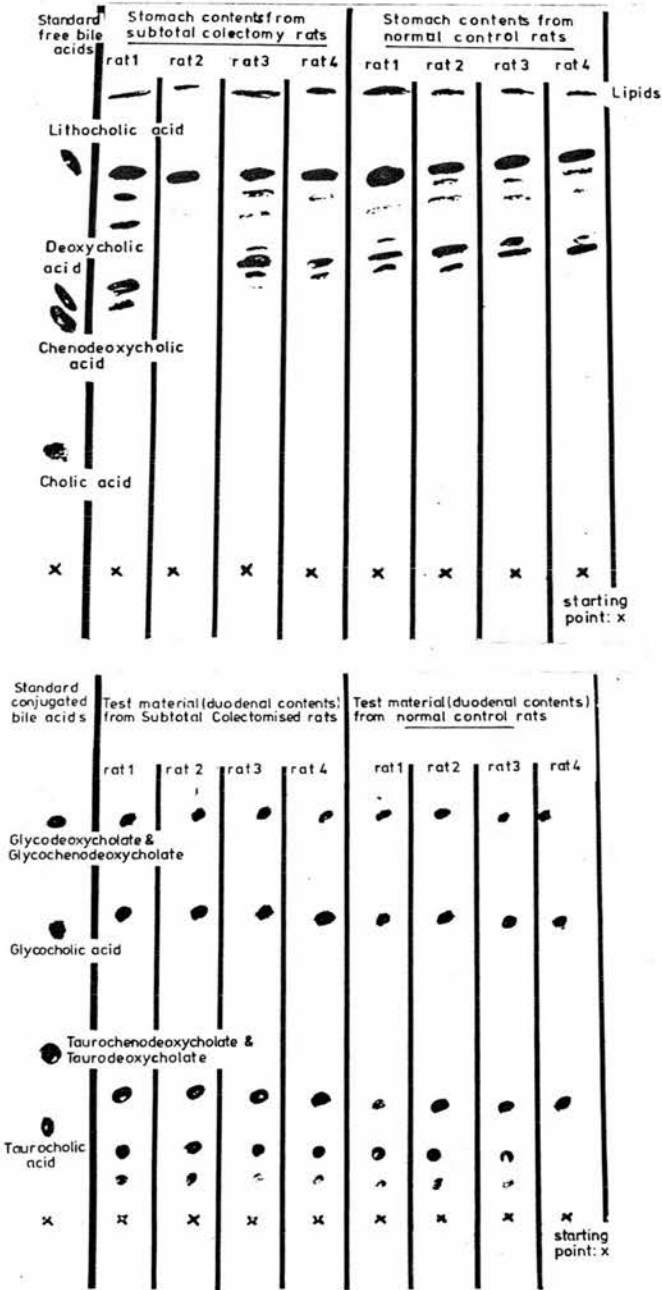
of  $8.4g \pm 0.3$ ;  $5.5g \pm 0.2$  and  $5.1g \pm 0.2$ , respectively. The difference between the output in subtotal colectomy and that in either control operated or unoperated rats was highly significant,  $p < 0.001$ .

Measurements taken during the third and fourth months after operation (for three days each time) revealed similar significant differences between the output in operated and control animals. Thus, at three months after surgery the daily faecal dry matter output for each operated, sham operated and unoperated rat was  $8.2g \pm 0.4$ ;  $5.2g \pm 0.2$  and  $4.9g \pm 0.3$ , respectively; and at four months it was  $7.9g \pm 0.2$ ;  $4.8g \pm 0.1$  and  $5.0g \pm 0.3$ , for subtotal colectomy, sham operated and unoperated rats, respectively.

Although faecal output was not measured in rats with colon bypass, by appearance the amount of faeces excreted by these animals was about the same as that passed by subtotal colectomy rats.

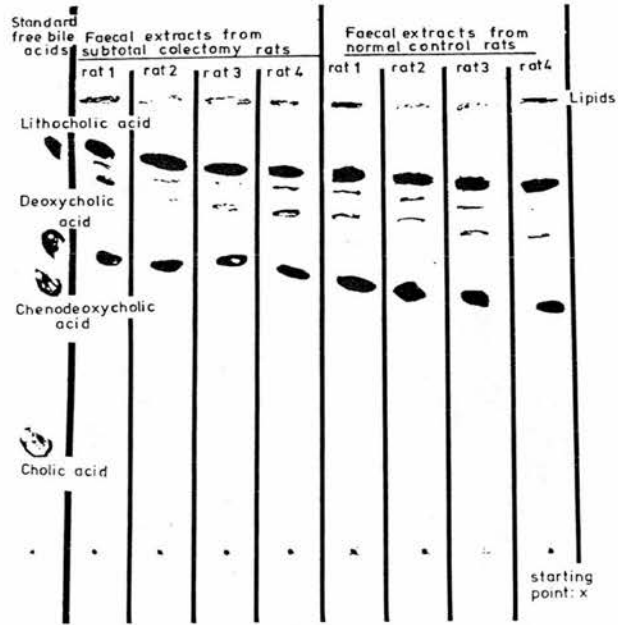
#### (b.i) Effect of Coprophagy on Faecal Output.

Throughout the 24-hours of collecting stools by the grab sampling technique, no faeces were passed between collections, or at least none were found on the trays nor in the cages, and the usual signs of coprophagy (bits and pieces of faeces dropped by the animal at eating) were absent. The average amount of faecal dry matter grabbed in 24 hours from each of 24 normal rats was  $5.6g \pm 0.2$ , with a range of 4.7g to 6.2g. These values are not significantly different from those obtained for 24-hour pooled stools collected as droppings from the same animals over 3 days, of which the mean daily output for each animal was  $5.3g \pm 0.2$  dry matter, the range being 4.5g to 5.9g. Thus, faeces collected by grab sampling exceeded the 24-hour pooled droppings by only about 5%, which would seem to indicate that insignificant amounts of stools were eaten by these animals during the sampling.



**Fig. 5a.**

Photo-copies of Thin-Layer-Chromatographs showing free bile acids in chloroform-methanol extracts of stomach contents (above) and conjugated bile acids in duodenal contents (below) taken from subtotal colectomised and normal control rats.



**Fig. 5b.**

Photograph of a Thin-Layer-Chromatograph showing free bile acids in chloroform-methanol extracts of faeces collected from subtotal colectomised and control rats.



(b.ii) Coprophagy After Subtotal Colectomy.

Analysis from the stomach contents of 8 subtotal colectomised and 8 unoperated rats by thin layer chromatography revealed the presence of free bile acids, particularly deoxycholic and lithocholic acids (Fig. 5a) in all samples examined three months after operation. Conjugated bile acids, which were present in extracts from duodenal contents, were not detected in any of the gastric samples. On the other hand, free bile acids similar to those detected in extracts from stomach contents were also found in faecal extracts (Fig. 5b). These findings strongly indicate that the free bile acids detected in gastric contents originated from faeces eaten by the animals, rather than from duodenal material regurgitated into the stomach. The occurrence of coprophagy (three months after surgery) is evident.

---

Discussion.

The absence of diarrhoea after left hemicolectomy seems to indicate that the distal large intestine in the rat does not play an important role in the absorption of water. This seems to be the case in the human colon, where in vivo measurement of sodium chloride and tritiated water transport (Levitan *et al.*, 1962) have indicated that water and electrolytes are predominantly absorbed from the right colon. This may explain why little diarrhoea results from resection of the left colon in man (Wangensteen and Minn, 1943; Gazet, 1964).

On the other hand, transient diarrhoea, occurring predominantly during the first two to three weeks after operation, developed in all right hemicolectomised rats used in this work. Such diarrhoea was observed by Lambert (1965) in 12 caeectomised rats; and commonly occurs

in man after resection of the right colon (Wangensteen and Minn, 1943; Gazet, 1964). The cause of this diarrhoea may involve other factors besides the loss of absorptive cell mass (or surface): in man, for example, colectomy with preservation of the ileocaecal valve reduces the incidence of loose stools (Lillehei and Wangenstein, 1955), even after extensive resections in which the caecum is anastomosed to the rectum (Rosi and Cahill, 1962; Webster and Howard, 1973); indicating that the ileocaecal valve plays an important part in ensuring adequate absorption from the small intestine by retarding the passage of digesta into the large bowel. This is supported by the following observations: 1) intestinal transit studies in the rat, using radioactive chromate (Nygaard, 1967b ; Johnasson and Nylander, 1968), have demonstrated temporary hold-up of ileal contents at the ileocaecal junction. In man, a barium sulphate meal reaches the end of the ileum about one hour before an appreciable quantity enters the caecum, and the ileum is often still full 4 or 5 hours after the last trace of barium has left the stomach (Ogilvie, 1931), indicating a delayed passage of bowel contents at the ileocaecal junction. 2) During radiological examination of dogs, Stahlgren et al., (1962) observed that resection or bypass of the ileocaecal junction significantly shortened the passage of barium sulphate through the small intestine. The stomach-to-anus transit of methylene blue was also greatly reduced after removal of the ileocaecal valve in dogs (Richardson, 1970). Also, in the present study the transit of barium sulphate through the small intestine was significantly shortened after right hemicolectomy (page 46.). 3) In man resections of the small intestine which include removal of the ileocaecal valve cause greater faecal loss of water and fat than similar resections in which the ileocaecal junction is left intact (Kalsler et al., 1960; Kiamo, 1969), indicating that the

temporary hold-up of bowel contents at the ileocaecal valve facilitates absorption from the small intestine by prolonging the duration of contact between bowel contents and mucosal surface. Hammer et al., (1959) provide some evidence in support of this: dogs in which 80% of the small intestine, including the ileocaecal junction, was removed, survived and maintained weight for over 2 years if 1-2 inches of the distal segment of the remaining bowel was reversed and reinserted into the intestine in an antiperistaltic manner (to serve as a substitute for the ileocaecal valve); animals with similar resections of the small bowel in which reversal of the intestine was not done developed severe diarrhoea and died of cachexia within three months. Repeated x-rays of the abdomen after a barium meal indicated that the chyme was held for more than 2 hours longer in the small intestine with reversed segment than in the small bowel without an ileocaecal substitute.

It has further been suggested that the ileocaecal valve ensures absorption by acting as a bacteriological barrier, preventing invasion of the small intestine by colonic micro-organisms (Richardson and Griffen, 1972; Gazet and Kopp, 1964). Thus, bile acid deconjugating anaerobacteria, such as bacteroides and clostridia (Drasar, Hill and Shiner, 1966; Norman and Grubb, 1955; Hill and Drasar, 1968) which commonly occur in large numbers in the faeces (Levine et al., 1968) have not been isolated from the small intestine of normal rats (Nygaard, 1967), or of normal man (Drasar, Hill and Shiner, 1966; Drasar and Shiner, 1969; Cregan and Hayward, 1953). On the other hand, experiments on dogs have shown that anastomosing the small intestine to the colon after excision of the distal small gut (Richardson, 1970) or the right colon (Gazet and Kopp, 1964) increases the bacterial flora of the small intestine, as judged from colony counts; resections which leave the ileocaecal valve

intact have no such effect. The increase in bacterial population has been shown by Richardson and Griffen (1972) to be a result of ascending overgrowth of colonic bacteria. Colonisation of the small intestine by colonic micro-organisms has also been observed in patients with jejunocolic fistula (Drasar et al., 1966), and is known to cause malabsorption, especially of fat (Tabaqchali and Booth, 1966; Gracey, 1971).

It has been postulated that colonisation of the small bowel by bile deconjugating bacteria causes a decrease in the level of conjugated bile acids (Tabaqchali, Hatzionnou and Booth, 1968) and a rise in the concentration of free bile acids within the lumen of the small intestine (Tabaqchali et al., 1968; Tabaqchali and Booth, 1966). If the concentration of conjugated bile acids is reduced to a level below the critical micellar concentration, and thus below the level necessary for normal fat digestion and absorption (Kim et al., 1966), diarrhoea may occur as a result of fat malabsorption. According to current belief diarrhoea in fat malabsorption is induced by faecal hydroxy fatty acids, which, incidentally, are excreted in increased quantities by patients suffering from fat malabsorption (Kellock et al., 1961; Kim and Spritz, 1968; James et al., 1961). The abundance of hydroxy fatty acids in steatorrhoea stools, and the observation that one of these fatty acids (stearic acid, a bacterial metabolite of oleic acid) has structural similarity to a known cathartic, ricinoleic acid, the active principle of castor oil, led to the suspicion that faecal hydroxy fatty acids induce diarrhoea; ricinoleic acid alters intestinal motility, increases mucus secretion, produces chemical gastroenteritis and also decreases sodium and water transport in vitro (Phillis, 1972). Perfusion studies in man (Amon and Phillips, 1972), in the rat (Brigh-Assare and Binder, 1973; Harries and Sladen, 1972) and in the hamster (Teem and Phillips, 1972)

have shown that hydroxy fatty acids inhibit water absorption in the jejunum, and cause water secretion in both the small and large intestine. In the colonised small bowel, it is thought that hydroxy fatty acids are produced by the action of colonic bacteria on unabsorbed long-chain triglycerides (Hofmann and Poley, 1972; Phillips, 1972).

In addition, since the major site for bile acid absorption in man (Borgstrom <sup>11</sup> et al., 1963), the dog (Playoust et al., 1965) and the rat (Barker and Searle, 1960) is the distal ileum, right hemicolectomy which includes removal of a large portion of the distal ileum is likely to cause bile acid malabsorption. This may lead to more bile acids reaching the remaining colon, where they may inhibit absorption (Mekhjian, Phillips and Hofmann, 1968; Mekhjian and Phillips, 1970) and induce secretion (Mekhjian and Phillips, 1971) of water and electrolytes, thereby causing diarrhoea. Malabsorption may also interrupt the enterohepatic circulation of bile acids, leading to decreased return of bile acids to the liver (Hofmann, 1967; Garbutt, Lack and Tyor, 1971), and increased rate of synthesis (Hofmann and Poley, 1972; Hofmann, 1967) with the result that the amount of bile acids reaching the large intestine is further increased, augmenting diarrhoea.

Following subtotal colectomy, a more severe watery diarrhoea developed in all the operated rats used in this work. Such diarrhoea was observed by Lambert (1965) and Wright et al. (1969b) in subtotal colectomised rats, and is commonly known to occur in man after similar bowel resections (Best, 1948; Best, 1952; Lillehei and Wangenstein, 1955; Hughes and Bennett, 1973). Excoriation of the skin developed as a complication following the frequent passage of corrosive ileal ejecta in most of the subtotal colectomised rats. This complication also occurs after the establishment of ileostomy in man (Louis et al., 1964).

Although more colonic absorptive surface is lost after subtotal colectomy than after right hemicolectomy, the occurrence of severe diarrhoea after the former operation may partly be due to other factors. Cummings et al., (1973), for example, studied six patients who had resection of 14-100% of the colon including the distal ileum; they observed that the more extensive the colon resection, the more rapid was transit of polyethylene pellets from stomach-to-anus; and the greater was the faecal output (wet weight). This would seem to indicate that the duration of contact between bowel contents and mucosal surface decreases with increase in the extent of colon resection, and may partly determine the severity of diarrhoea.

In the present study, although the transit of barium sulphate from stomach-to-anus was greatly speeded after subtotal colectomy (page 47.), there was no evidence to suggest a reduced duration of contact between chyme and small bowel mucosal surface; so the occurrence of severe diarrhoea would seem to be a result of the greater loss of colonic absorptive surface.

Faecal output and faecal water content were studied on the basis that increased faecal dry matter output without concomitant increase in food intake is suggestive of impaired digestion; and that changes in faecal water content indicate changes in intestinal absorptive ability.

The 24-hour grab collection of faeces showed that insignificant quantities of faeces were eaten by the rats used in this study, so that the increase in faecal output in operated animals is unlikely to have been caused by absence of coprophagy. However, it must be stressed that it is not certain whether timed grab sampling completely prevented coprophagy; it is possible that an appreciable amount of faeces might have been taken directly from the anus and eaten by the normal rat

without any being dropped. Barnes et al. (1957) observed that when rats were completely prevented from eating their faeces (by collecting all faeces as they were passed directly into containers specially designed to be fixed on the tail to cover the anus) the faecal dry matter output increased by 50 to 60%. Lutton and Chevallier (1973) also report that rats eat 41% of the pellets they produce. In the present study, faeces collected by grab sampling exceeded those collected as 24-hour pooled droppings by only 5%.

The occurrence of coprophagy in subtotal colectomy rats demonstrated (three months after operation) by the presence of free bile acids in stomach contents further indicates that the increase in faecal dry matter output in operated animals is unlikely to have been caused by absence of coprophagy, although there is still a possibility that eating of faeces might be less marked in operated animals, because of change in the consistence of the stools.

Since food intake of right hemicolectomised rats was not increased, the increase in faecal output in these animals would seem to indicate impaired digestion. It is most likely that removal of the caecum impairs the fermentative processes which normally occur in the rat's large intestine and degrade dietary cellulose to volatile fatty acids (Yang et al., 1969). In such a situation the cellulose present in the diet would pass undigested into the stools, thereby increasing the amount of excreted faecal dry matter. Decreased digestibility of normal rat diet has been demonstrated in caeectomised rats (Dermane et al., 1973).

In the present study determination of energy content per gram of dry faeces, by bomb calorimetry (page 34.) provided results which do not indicate any marked impairment of digestion after subtotal colectomy. However, since bacterial activity was not arrested, fermentation could

have continued between collection and analysis of the samples; therefore, the results obtained in this case may not reflect the true energy content in the faeces, and the state of digestive ability. The fact that after three weeks of pair feeding, subtotal colectomised rats (three months post-operatively) lost on average 4% of their weights at the beginning of pair feeding, despite a food intake which was associated with normal body weight gain in the control animals would seem to indicate faulty absorption of dietary energy, presumably resulting from impaired digestion. Following loss of the large intestine about 5% of the daily energy intake derived from a normal rat diet would be unavailable to the rat (Yang *et al.*, 1970), which would probably be sufficient to account for the loss of weight seen in the pair-fed subtotal colectomised rat (page 42.).

Although the increase in food intake in subtotal colectomised rats seems partly to account for the high faecal dry matter output, it would appear that the main cause of increased output was impaired digestion: at three months after operation the food intake per rat/24 hours, measured during 7 days before pair feeding was started, averaged  $18.6 \pm 0.7\text{g}$  and  $25.8 \pm 0.6\text{g}$  for sham operated and subtotal colectomised rats, respectively. The corresponding mean faecal dry matter output per rat/24 hours was  $8.1 \pm 0.3\text{g}$  and  $4.9 \pm 0.4\text{g}$  for operated and control rats, respectively, being about 60% more in the subtotal colectomy group. On pair feeding operated animals continued to pass 36% more faeces than control animals. It would appear, therefore, that the extra food consumed by subtotal colectomised rats during ad libitum feeding contributed to the increase in faecal dry matter output simply because of decreased digestibility of the diet.

The present findings, therefore, seem to indicate impairment of digestion as being the cause of increased faecal dry matter output both



Table 2

Food Intake, and body weight, in Right Hemicolectomised (RH), Sham Operated (SO) and Unoperated (UC) rats :

weeks before surgery.	duration of study (days)	animal group	mean body wt. (grams) - s.e.m.	24-hour Food Intake (dry mat.)	
				grams/rat. mean $\pm$ s.e.m.	grams/100g body wt. mean $\pm$ s.
(age:) 3	7	UC	270 $\pm$ 9	19.5 $\pm$ 0.9	7.2 $\pm$ 0.1
( 13 ) 3	7	RH	282 $\pm$ 10	19.7 $\pm$ 1.4	7.0 $\pm$ 0.2
(wks.) 3	7	SO	289 $\pm$ 8	21.7 $\pm$ 1.0	7.4 $\pm$ 0.2
	2	UC	299 $\pm$ 7	21.5 $\pm$ 0.9	7.3 $\pm$ 0.1
	2	RH	305 $\pm$ 8	21.1 $\pm$ 1.3	6.9 $\pm$ 0.2
	2	SO	319 $\pm$ 8	21.0 $\pm$ 2.1	6.8 $\pm$ 0.1
	1	UC	320 $\pm$ 9	21.3 $\pm$ 0.3	6.7 $\pm$ 0.1
	1	RH	328 $\pm$ 9	21.9 $\pm$ 0.5	6.5 $\pm$ 0.4
	1	SO	341 $\pm$ 9	21.7 $\pm$ 1.4	6.5 $\pm$ 0.1
weeks after surgery.					
	2	UC	381 $\pm$ 7	20.5 $\pm$ 0.2	5.4 $\pm$ 0.2
	2	RH	347 $\pm$ 9	20.9 $\pm$ 0.6	6.1 $\pm$ 0.2
	2	SO	380 $\pm$ 9	19.9 $\pm$ 0.5	5.4 $\pm$ 0.2
	7	UC	415 $\pm$ 9	20.1 $\pm$ 0.6	5.2 $\pm$ 0.2
	7	RH	398 $\pm$ 9	21.1 $\pm$ 1.6	5.4 $\pm$ 0.1
	7	SO	436 $\pm$ 9	21.9 $\pm$ 0.9	5.0 $\pm$ 0.2

n = 8 in each animal group.

after right hemicolectomy and subtotal colectomy. The decrease in faecal water content 2 months after right hemicolectomy, and the disappearance of diarrhoea, indicate improved water absorption in the adapted hemicolectomised and subtotal colectomised rats.

---

(c) Food Intake.

(c.i) After Right Hemicolectomy.

As shown in table 2, the food intake was approximately the same in right hemicolectomised, sham operated and unoperated rats. In all groups the amount of food consumed per 100g of body weight per day decreased with increase in body weight.

(c.ii) After Left Hemicolectomy.

Food consumption was the same in operated as in control animals: 2 months after operation the mean intake per rat per 24 hours, measured for 8 operated and 8 sham operated animals (over 7 days) was  $20.3 \pm 0.5g$  and  $19.6 \pm 0.7g$ , respectively (body weights being not significantly different).

(c.iii) Following Subtotal Colectomy.

The daily amount of food consumed by each rat was greatly increased after subtotal colectomy (Table 3.). Two weeks after operation the mean food intake (grams/100g of body weight per 24 hours) was about 33% greater in operated rats than in either sham operated or unoperated controls. This increased further to about 40% above control values, three and four months after surgery.

Table 3.

Food Intake, and body weight, in Subtotal Colectomy (SC) Sham operated (SO),  
and Unoperated (UC) Rats.

when studied.	animal group.	body wt(g). mean $\pm$ s.e.m.	24-hour Food Intake (dry food matter)	
			grams/rat mean $\pm$ s.e.m.	grams/100g body wt. mean $\pm$ s.e.m.
2 weeks after operation.	SC	299 $\pm$ 12	23.8 $\pm$ 0.7	7.9 $\pm$ 0.2
	SO	332 $\pm$ 20	19.3 $\pm$ 0.6	5.8 $\pm$ 0.1
	UC	308 $\pm$ 12	19.6 $\pm$ 0.5	6.3 $\pm$ 0.1
3 months after operation	SC	395 $\pm$ 18	26.1 $\pm$ 0.6	6.7 $\pm$ 0.2
	SO	421 $\pm$ 11	20.3 $\pm$ 0.4	4.8 $\pm$ 0.1
	UC	418 $\pm$ 15	19.5 $\pm$ 0.5	4.6 $\pm$ 0.1
4 months after operation	SC	418 $\pm$ 19	24.8 $\pm$ 0.9	5.9 $\pm$ 0.2
	SO	470 $\pm$ 18	18.5 $\pm$ 0.9	4.0 $\pm$ 0.1
	UC	460 $\pm$ 19	19.1 $\pm$ 0.5	4.3 $\pm$ 0.2

In each group n = 8. Food intake was studied during 5-day periods and was found to be significantly greater in subtotal colectomised rats than in either control group,  $p < 0.01$  in each case.

Table 4.

Energy equivalent of food ingested and faeces.

	SC	SO	pair t-t
food intake: dry matter, (g)/rat/24hours, mean $\pm$ s.e.m.	24.3 $\pm$ 0.9	17.8 $\pm$ 0.3	p <
energy content in diet: kcal/gram of dry matter, mean $\pm$ s.e.m.	4.26 $\pm$ 0.01	4.26 $\pm$ 0.01	
energy equivalent of food ingested kcal/rat/24hours, mean $\pm$ s.e.m.	102.23 $\pm$ 2.93	74.76 $\pm$ 4.94	p <
faecal dry matter output/rat/ 24hours, (g); mean $\pm$ s.e.m.	7.7 $\pm$ 0.3	4.8 $\pm$ 0.6	p <
energy content in faeces, kcal/gram of dry faeces: mean $\pm$ s.e.m.	3.83 $\pm$ 0.04	3.77 $\pm$ 0.05	
energy equivalent of faeces excreted per day/rat: kcal, mean $\pm$ s.e.m.	28.24 $\pm$ 1.13	18.04 $\pm$ 0.68	p <
energy intake - energy output = energy absorbed: kcal/rat/24 hours, mean $\pm$ s.e.m.	73.99 $\pm$ 2.17	56.81 $\pm$ 5.23	p <
energy absorbed as % of energy intake, mean $\pm$ s.e.m.	72.3 $\pm$ 0.7%	76.0 $\pm$ 2.2%	p >

(c.iv) Rats with subtotal colon bypass consumed, on average, approximately the same amounts of food as subtotal colectomised animals. Thus, at 14 weeks after operation the daily intake per rat, measured over 5 days for 8 colectomy, 8 colon bypass and 8 sham operated rats was  $22.6g \pm 0.6$ ,  $23.0g \pm 0.4$  and  $18.9g \pm 0.3$ , respectively.

(c.v) Dietary Energy Absorption.

The daily dietary energy absorption, as determined from the difference between the energy equivalent of the food ingested and the faeces excreted, was studied in 6 subtotal colectomised (SC) and 6 sham operated (SO) rats. Results are presented in table 4.

---

Discussion.

While control and adapted hemicolectomised rats spent most of the day time sleeping, animals with subtotal colectomy or colon bypass quite often indulged in eating during this period, and it would appear that the hyperphagia which developed after subtotal colectomy or colon bypass was a result of increased feeding frequencies.

Regulation of food intake both in man and animals seems to be brought about by the interaction of many factors which modify the activity of the food intake control mechanism in the hypothalamus. Studies in animals, in which electrolytic lesions were produced in different regions of the hypothalamus, have established the existence of a dual control mechanism consisting of a lateral feeding centre which provides the basic urge to eat (Anand and Brobeck, 1951a; Anand and Brobeck, 1951b; Anand et al., 1955), and a medial satiety centre which acts by inhibiting the lateral mechanism (Hetherington and Ranson, 1940; Hetherington and Ranson, 1942; Brooks et al., 1942; Anand et al., 1955; Mayer et al., 1955).

Various suggestions have been made regarding the trigger mechanism for the urge to eat, and the nature of changes, or changes, produced as a result of feeding, which signal to the regulating system that further feeding should be stopped:

1) It has been postulated that stimulation of "head receptors" by smelling, tasting, chewing and swallowing, during eating, plays an important role in bringing about satiety and suppression of further eating (Grossman, 1955), but that this factor is relatively ineffective when it is not associated with entry of food into the stomach: thus, if a dog is allowed to eat a portion of its food a short time (not sufficient to allow absorption) before food is supplied *ad libitum*, the voluntary intake is reduced by an amount approximately equal to the prefeeding; but if instead of allowing the dog to eat the portion of food, the food is placed in the stomach just before food is offered *ad libitum*, voluntary intake is reduced to a lesser degree (Janowitz and Grossman, 1951). Conversely, in dogs with esophagostomy, sham feeding, in which the food fails to enter the stomach, results in the taking of greater amounts of food than in intact animals (Janowitz and Grossman, 1949; Share, Martyniuk and Grossman, 1952).

2) Distension of the stomach with the ingested food influences the quantity of food intake: this has been demonstrated by the inhibitory effect of gastric distension, e.g. with water-filled balloons, on the duration of sham-feeding in esophagostomised dogs (Janowitz and Grossman, 1949; Share et al., 1952) and by the production of sustained depression of food intake when water-filled balloons are placed in the stomach of dogs and kept there for weeks (Share et al., 1952). Electroencephalographic recordings (through stereotaxically implanted electrodes) of the electrical activity of the hypothalamic satiety and feeding centres taken

during distension of the stomach with water-filled balloon system (Sharman et al., 1961) have shown that gastric distension selectively increases the electrical activity in the region of the satiety centre, indicating inhibition of the urge to feed. On the other hand, since animals with complete denervation (vagotomy and sympathectomy) of the gastrointestinal tract show normal regulation of food intake (Grossman, Cummins and Ivy, 1947) it would appear that either the gastric distension mechanism is dispensable or that gastric distension may operate through somatic nerves stimulated by increases in the volume of the abdominal contents.

3) The glucostatic regulation of food intake hypothesis (Mayer, 1952; Mayer, 1953; Mayer, 1955; Mayer and Marshall, 1956) postulates the existence of glucoreceptors in the hypothalamus which are sensitive to the rate at which glucose is utilised by them, such that low utilization rates excite neural activity leading to hunger sensation and food-taking, whereas high utilization rates produce the opposite effect. Arteriovenous glucose differences serve as an index of utilization rate and have been shown to correlate with the urge to eat in normal man; when the A-V glucose difference is greater than 15mg/100 ml hunger is not felt, but persistent low differences are invariably accompanied by the urge to eat (Van Itallie, Beaudoin and Mayer, 1953), and submaintenance caloric intake is associated with small A-V glucose differences and more frequent hunger sensation. Further support for the glucostatic hypothesis is provided by the observations: that hunger sensation in man may promptly be abolished by intravenous glucose (Stunkard and Wolff, 1954); subcutaneous injections of glucose (1ml of a 37.5% glucose solution twice daily for 23 days) reduced food intake in normal rats by 10% (Mayer and Bates, 1952). However, Grossman (1955) reported experiments in which intravenous glucose leading to hyperglycaemia with elevation of A-V glucose difference did not

significantly depress hunger sensation, appetite or food consumption in normal man.

The existence of <sup>a</sup> glucoreceptor mechanism in the hypothalamic food intake regulating centres has been indicated by the finding that with hyperglycaemia, electroencephalographically recorded activity of the satiety centres in anaesthetised dogs and cats (Anand et al., 1961; Anand et al., 1964) increases, with some diminution in the activity of the feeding centre, while insulin induced hypoglycaemia produces the opposite effect. It has also been shown that the activity of the satiety centres selectively increases after a meal; and that this correlates well with increased A-V glucose difference (Anand et al., 1961).

4) It has further been suggested that because the amount of endogenous fat (in animals) mobilised daily in ad libitum feeding conditions seems to be proportional to the size of fat depots, i.e. a constant proportion of the body fat may be mobilised daily (Bates, Mayer and Nauss, 1955), the mobilised fat plays a role in regulating both the body weight and food intake, an increase in fat content being followed by increased availability of readily utilizable fat, with consequent sparing effect on carbohydrates (Mayer, 1955). Because of the interrelationship of carbohydrate and fat metabolism, the above lipostatic hypothesis could be integrated with the glucostatic mechanism to provide a long-term regulation of food intake.

5) Other factors thought to influence the amount of food intake in man and animals include: changes in environmental temperature, low temperature being associated with increased food intake, while high temperatures cause reduced food intake (Brobeck, 1948; Brobeck, 1955); the concentration of circulating proteins and amino acids; excessively high protein content of the diet and imbalance or excess of individual amino

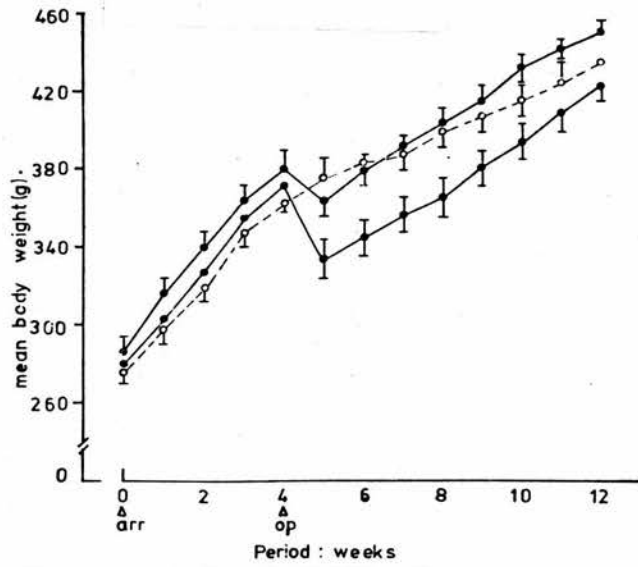


acids in a low-protein diet being associated with reduced food intake (Kraus and Mayer, 1965); and the water concentration in the diet (Strominger, 1944).

In the present study the degree of impairment of digestion, as indicated by the increase in faecal dry matter output, was about the same after right hemicolectomy as after subtotal colectomy. Thus, in animals of similar body weights and age, eating approximately the same quantities of food per day (i.e. right hemicolectomised and sham operated rats on the one hand, and subtotal colectomised and sham operated rats during pair feeding on the other) it was found that the average faecal output in right hemicolectomised and subtotal colectomised animals exceeded that in controls by 30% and 36% respectively. Also, when the amount of faeces excreted per day, in animals of comparable body weights under ad libitum feeding conditions, was expressed as a percentage of the food intake, unoperated, right hemicolectomised and subtotal colectomised rats excreted 24%, 30% and 32%, respectively, of their food intake, which further indicates that the degree of impairment of digestion after either right or subtotal colectomy was about the same. The hyperphagia that developed after subtotal colectomy or colon bypass, therefore, would seem unlikely to have been induced by energy deficit resulting from impaired digestion alone. However, decreased digestion of the diet is likely to have reduced the amount of available dietary energy, so that consumption of normal amounts of food resulted in submaintenance caloric intake both in hemicolectomised and subtotal colectomised rats. The slight increase in food intake after right hemicolectomy, although not statistically significant, probably compensated for the energy deficit. Also the adaptive change in the gastrointestinal motility associated with prolongation of small bowel emptying-time (page 47) probably ensured a more complete absorption of the available energy.

On the other hand, the severe diarrhoea that invariably developed in all rats with subtotal colectomy could have been associated with malabsorption of the available dietary energy. Although measurement of the energy equivalent of the food ingested and the faeces failed to indicate marked malabsorption, the fact that subtotal colectomised rats lost weight during the pair-feeding period (page 42. ) shows that these animals were not able to obtain from a normal food intake sufficient energy to maintain both the high postoperative metabolic rates and normal growth. After major tissue injury, e.g. limb fractures in man (Cuthbertson, 1932), there is marked increase in metabolic activities (indicated by high oxygen consumption, increased urinary nitrogen excretion and depletion of body proteins, raised body temperature, pulse rate and increased food intake) during the first week of injury; these may remain high throughout the inflammatory (defence and demolition) period, which may last for a considerable time, depending on the severity of injury (Cuthbertson, 1959).

In the rat, the compensatory increase in intestinal mucosal mass (pages 52, 60. ) can only be achieved through increased metabolic activity; the greater the compensatory work the more would be the energy requirement. Thus, high energy expenditure, together with a submaintenance intake, may have caused the hyperphagia in subtotal colectomised rats; this could be effected through the glucostatic and/or lipostatic mechanism. In man, hyperphagia, i.e. eating 5,000 - 11,000 kcal/day, has sometimes been observed in patients with malabsorption, indicated by glucose tolerance tests and steatorrhoea (Hall and Creamer, 1974). This hyperphagia was also probably a result of energy deficit occurring with a normal food intake. It is also likely that water and electrolyte imbalance (resulting from the watery diarrhoea in subtotal colectomised rats) might play a contributory role in increasing food intake (Brobeck, 1955; Strominger, 1944).



**Fig. 6.** Body weight (mean  $\pm$  s.e.m.) of rats before and after operation (op). Sham operated: top trace with closed circles; right hemicolectomy, lower trace with closed circles; unoperated, open circles. 'arr' means arrival at 3 months of age. For each circle  $n = 8$ .

The other possibility is that subtotal colectomised rats might eat little of their ill-formed faeces, so that with reduced coprophagy they eat more food instead.

---

(d) Body Weight.

(d.i) After Right Hemicolectomy.

At the end of the first week after surgery, right hemicolectomised and sham operated rats had lost on average  $12.1 \pm 1.0\%$  and  $5.9 \pm 0.9\%$ , respectively of their preoperative body weights, the loss being significantly greater in the hemicolectomised than in sham operated rats,  $p < 0.01$ . However, after this period both operated and sham operated rats continued to gain weight steadily (Fig. 6), although at each time point between the first and fifth postoperative week operated rats remained significantly underweight when compared to controls,  $p < 0.01$  each time. After the fifth week 't' tests failed to reach significance, although mean control values remained above mean colectomy weights (Fig. 6).

Body weight gain was approximately the same after right hemicolectomy and sham operation; at the end of the first postoperative week the mean body weight for hemicolectomised, sham operated and unoperated rats was  $335 \pm 6g$ ,  $364 \pm 8g$  and  $376 \pm 4g$  respectively; the corresponding weights at killing (8 weeks after surgery) were  $424 \pm 7g$ ,  $452 \pm 6g$  and  $438 \pm 3g$  for hemicolectomy, sham operated and unoperated rats, respectively. Thus, at killing hemicolectomised and sham operated animals had gained, on average,  $26.5 \pm 1.6\%$  and  $24.4 \pm 3\%$ , respectively, of their body weights at the end of the first postoperative week. On the other hand, during the same period, unoperated rats increased in weight by  $16.4 \pm 2.7\%$ , this

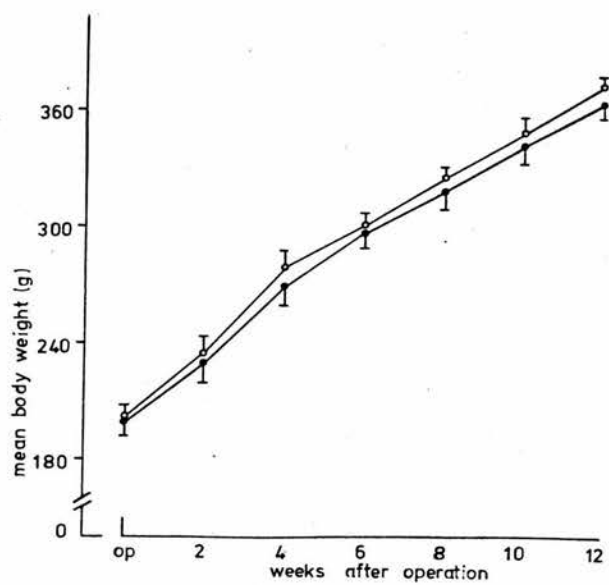


Fig. 7.

Body weight (mean  $\pm$  s.e.m.) after left hemicolecotomy, closed circles, and sham operation, open circles. Age of animals at operation (op): 8 weeks.

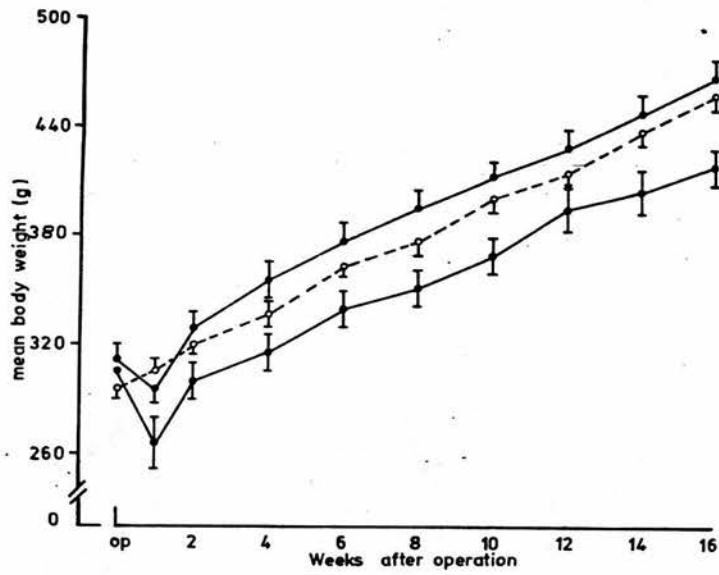


Fig. 8.

Body weight (mean  $\pm$  s.e.m.) of subtotal colectomised rats (bottom graph), sham operated (top graph) and unoperated (middle graph) controls. Age of animals at operation (op): 15 weeks. For each circle  $n = 8$ .

being significantly less than the body weight gain in either hemicolectomised or sham operated rats,  $p < 0.01$  in either case.

(d. ii) Body Weight after Left Hemicolectomy.

Throughout the study period the mean body weight in operated rats remained approximately the same as that in control animals (Fig. 7). At three months after operation left hemicolectomised rats had gained, on average,  $91.8 \pm 1.0\%$  of their preoperative weights, while sham operated controls gained, over the same period,  $95.4 \pm 1.9\%$ .

(d.iii) Following Subtotal Colectomy.

The average loss of weight at the end of the first week after surgery in a group of 8 operated and 8 sham operated rats amounted to  $13.6 \pm 0.7\%$ , and  $5.2 \pm 0.3\%$ , respectively, of the preoperative body weight. The loss was significantly greater in operated than in control animals,  $p < 0.025$ . Although rats with subtotal colectomy, when compared to sham operated controls, remained underweight throughout a 4-month study period (Fig. 8), the rate of body weight gain was approximately the same in the two groups. Thus, at the end of the fourth postoperative month, operated and sham operated rats had gained, on average,  $54.6 \pm 1.3\%$  and  $58.4 \pm 1.6\%$ , respectively, of their body weights at 1 week after surgery. The corresponding weight gain in unoperated controls, over the same period, was  $52.8 \pm 1.8$ .

(d.iv) After Colon Bypass.

There was no significant difference between the body weights of rats with colon bypass and those with subtotal colectomy (Table 5). In this group animals were operated on at two months of age, and the postoperative loss of body weight was less marked in both colon bypass and colectomised animals; all operated animals in this group grew at approximately the

Table 5.

Body Weight of Subtotal Colectomised (SC), Colon Bypass (CB) and Unoperated (UC) rats.

when studied	UC (n=8)		SC (n=8)		CB (n=8)	
	(g):range.	(g): mean $\pm$ s.e.m.	(g):range.	(g):mean $\pm$ s.e.m.	(g):range.	(g):mean $\pm$ s.e.m.
at operation (age: 8 wks)	247-300	260 $\pm$ 7	250-280	263 $\pm$ 7	250-300	265 $\pm$ 9
2 weeks after surgery.	263-355	302 $\pm$ 5	240-310	285 $\pm$ 8	352-332	297 $\pm$ 6
6 weeks after surgery.	290-400	356 $\pm$ 9	275-379	327 $\pm$ 10	311-385	348 $\pm$ 10
10 weeks after surgery.	320-457	399 $\pm$ 7	300-443	384 $\pm$ 9	330-456	391 $\pm$ 9
14 weeks after surgery.	390-487	428 $\pm$ 9	365-463	411 $\pm$ 12	368-478	420 $\pm$ 11



Table 6.

Body weight and food intake in subtotal colectomised (SC) and sham operated (SO) rats. Pair fed group.

when studied.	<u>Mean body weight (g) <math>\pm</math> s.e.m.</u>		<u>Mean food intake (g) <math>\pm</math> s.e.m. per rat per 24 hours.</u>		Paired t-test
	(SO) n=8.	(SC) n=8.	(SO) n=8.	(SC) n=8.	
1 week before surgery	191 $\pm$ 7	191 $\pm$ 9	----	----	
2 weeks after surgery	233 $\pm$ 9	222 $\pm$ 12	20.2 $\pm$ 0.7	26.0 $\pm$ 0.6	p 0.0
5 weeks after surgery	405 $\pm$ 11	382 $\pm$ 18	20.8 $\pm$ 0.9	25.3 $\pm$ 0.8	p 0.0
11 weeks after surgery	447 $\pm$ 10	428 $\pm$ 13	18.6 $\pm$ 0.7	25.8 $\pm$ 0.6	p 0.0
12 weeks after surgery	453 $\pm$ 11	424 $\pm$ 10	18.4 $\pm$ 0.5	18.4 $\pm$ 0.5	(pair
13 weeks after surgery	456 $\pm$ 8	418 $\pm$ 14	18.4 $\pm$ 0.7	18.4 $\pm$ 0.7	(pair
14 weeks after surgery	464 $\pm$ 9	411 $\pm$ 15	18.6 $\pm$ 0.4	18.6 $\pm$ 0.4	(pair

\* Difference between food intake in operated and control ra

Table 7.

Blood picture in the rat after Subtotal colectomy (SC) or Sham operation (SO). All values mean  $\pm$  s.e.m.

when studied	animal group	total WBC count: $\times 10^3$ mm <sup>-3</sup>	RBC count: $\times 10^6$ mm <sup>-3</sup>	M.C.V. $\mu$ m <sup>3</sup>	Hct. %	Hb. grams:	M.C.H. (pg)	M.C.H.C. %
2 weeks after operation.	SC	17.3 $\pm$ 1.9	6.9 $\pm$ 0.2	58.2 $\pm$ 1.6	40.0 $\pm$ 1.2	14.4 $\pm$ 0.5	20.8 $\pm$ 0.3	36.0 $\pm$ 0.4
	SO	13.8 $\pm$ 1.0	7.6 $\pm$ 0.2	56.7 $\pm$ 1.1	42.7 $\pm$ 0.6	15.5 $\pm$ 0.4	20.3 $\pm$ 0.2	36.4 $\pm$ 0.6
4 weeks after operation.	SC	15.7 $\pm$ 0.9	7.8 $\pm$ 0.3	58.6 $\pm$ 0.7	42.3 $\pm$ 0.8	15.0 $\pm$ 0.4	20.7 $\pm$ 0.2	35.5 $\pm$ 0.2
	SO	16.4 $\pm$ 1.0	7.5 $\pm$ 0.2	57.4 $\pm$ 0.3	42.8 $\pm$ 0.9	15.4 $\pm$ 0.3	20.5 $\pm$ 0.1	36.0 $\pm$ 0.2
12 weeks after operation.	SC	15.4 $\pm$ 1.2	7.3 $\pm$ 0.2	57.9 $\pm$ 0.9	42.1 $\pm$ 0.9	15.0 $\pm$ 0.4	20.6 $\pm$ 0.3	36.8 $\pm$ 0.4
	SO	13.4 $\pm$ 1.2	8.1 $\pm$ 0.2	56.6 $\pm$ 0.4	43.8 $\pm$ 0.7	15.6 $\pm$ 0.3	19.3 $\pm$ 0.3	35.5 $\pm$ 0.3

n = 8 in both groups each time.

same rate as unoperated controls (Table 5).

(d.v) Body Weight of Subtotal Colectomised Rats Following Pair Feeding.

Although food intake was markedly increased in subtotal colectomised rats the rate of body weight gain was not significantly greater than in control animals. As shown in table 6, subtotal colectomised animals continued to gain weight on a food intake 30% greater than normal, but when offered approximately the same amount of food as that eaten by control animals, colectomised rats lost, within three weeks, an average of 4% of their body weight at the beginning of pair feeding. On the other hand, control animals increased in weight by 3.5% over the same period, taking the same quantities of food.

(e) Blood.

Examination of tail blood carried out in 8 subtotal colectomised and 8 sham operated rats at 2, 4 and 12 weeks after surgery failed to detect any abnormality determinable by the indices presented in table 7.

---

Discussion.

The present results indicate that left hemicolectomy has no significant influence on the body weight of adult rats. On the other hand, right hemicolectomy causes marked loss of body weight in the early postoperative period, but with recovery, the animals seem to gain more weight in a given period than controls. Since control animals subjected to sham operation also showed a greater rate of body weight gain than unoperated controls, the stimulus for increased growth is probably the initial loss of weight operating through an unknown mechanism.

All rats subjected to right hemicolectomy, subtotal colectomy or subtotal colon bypass showed no outward signs of malnutrition or avitaminoses. After the early preoperative diarrhoea, animals became active and remained well groomed; macrocytic anaemia, which has been reported in caeectomised rats (Plum, 1950), did not develop by the third month after subtotal colectomy. These observations seem to indicate that loss of the large intestine in the adult rat leads to no significant nutritional deficiencies. However, this is probably true only when the animals are maintained on a diet rich in vitamins. Previous studies involving measurement of vitamin content in intestinal juices, secured from different parts of the digestive tract of intact and caeectomised animals, have established that important synthesis of vitamin K (Mickelsen, 1956) and of vitamin B complex (McGregor et al., 1947; Johnsson et al., 1953) occurs in the large intestine, particularly the caecum, of rats. So that intact normal rats maintained on a diet free from these vitamins grow normally, without developing signs of avitaminosis, as long as they are allowed to eat their faeces (Barnes and Fiala, 1958; Barnes, Kwong and Fiala, 1959; Barnes and Fiala, 1959). Although resection of the caecum seems to impair intestinal synthesis of vitamins (Guerrant et al., 1935; Day et al., 1943; Schweigert et al., 1945), caeectomised young rats maintained on a well balanced stock diet grow normally (Griffith, 1935; Taylor et al., 1942; Lambert, 1965), but lose weight when fed on a diet deficient in vitamin B complex (Guerrant et al., 1935).

In the present study, there were added vitamins in the rat diet, so that failure to grow and thrive through vitamin deficiency was unlikely. However, the fact that rats subjected to right hemicolectomy or subtotal colectomy, particularly those operated on after three months of age, tended to remain underweight when compared to control animals, is an

indication that the colon in this animal plays an important role in the maintenance of body weight (or growth). But since both these operations include removal of the ileocaecal valve as well, the part played by loss of the colon per se cannot be assessed; Gazet and Kopp (1964) studied the effect of selective ablation of either the ileocaecal junction or the proximal half of the large intestine in rabbits, cats, dogs and one Rhesus monkey, and observed that an equivalent loss of weight resulted from either operation. Except for the monkey, which regained its postoperative body weight by the 55th day after surgery, all other animals, whether subjected to hemicolectomy or ileocaecal resection, failed to regain their preoperative weights by the 105<sup>th</sup> day after surgery.

The occurrence of hyperphagia, and the loss of body weight during pair feeding, in subtotal colectomised rats, are further indications that a significant amount of ingested energy is made available to the animal by the presence of the large intestine. Animals consumed more food than normal and thus compensated for the energy deficit caused by removal of the colon; when food intake was restricted by pair feeding, subtotal colectomised rats lost weight; presumably because they were not able to derive from a normal food intake sufficient energy to maintain normal growth. It would seem, therefore, that the large intestine, at least in the rat, plays an important role in the maintenance of normal growth.

The age at which surgery is performed seems to determine the postoperative body weight loss and the subsequent growth. Animals operated on between the age of 2 and 3 months, as in (d.ii) and (d.iv) above, show less marked postoperative body loss and subsequently maintained approximately the same body weight as unoperated controls, even after they have been subjected to subtotal colectomy. On the other hand, rats operated on at a later age lose much more weight in the early postoperative period and

remain underweight when compared to control animals, unless they are able to compensate for the initial weight deficit by establishing, and maintaining for a long period, a growth rate higher than normal.

---

PART 4.CHANGES IN STRUCTURE AND FUNCTION.(a) Intestinal Transit Time.(a.i) After Right Hemicolectomy.

Transit of barium sulphate (3ml of Micropaque) through the small intestine was studied in 6 right hemicolectomised rats (three months postoperatively) and in 6 unoperated controls. At the time of study all the animals involved weighed approximately the same, and were of the same age.

The stomach-to-caecum transit time in control rats, as judged from the time taken by the head of the barium colon to reach the caecum, averaged 220 minutes, with a range of 175-295 minutes. In the hemicolectomised animal, barium reached the colon in 145-205 minutes, the average being 177 minutes. Although the meal took about 40 minutes less to traverse the small intestine in operated rats, the colon still contained little barium for a much longer time than the caecum in control animals: at 273 minutes (range, 265-290), the caecum contained most of the meal, while the colon in operated animals contained little barium until 345 minutes (range, 310-400 minutes) after introduction of the meal; t-test showed significant shortening of small bowel transit time in the hemicolectomised animals,  $p < 0.01$ . Also, the time taken by the major portion of the meal to enter the colon in operated rats was significantly longer than that taken by barium to enter the caecum in control animals,  $p < 0.01$ .

As would be expected from the above, the meal took a longer time to be completely evacuated from the small intestine of operated animals, the mean small bowel emptying time being 430 minutes (range, 385-450) and 493 minutes (range, 450-510) for control and operated animals,

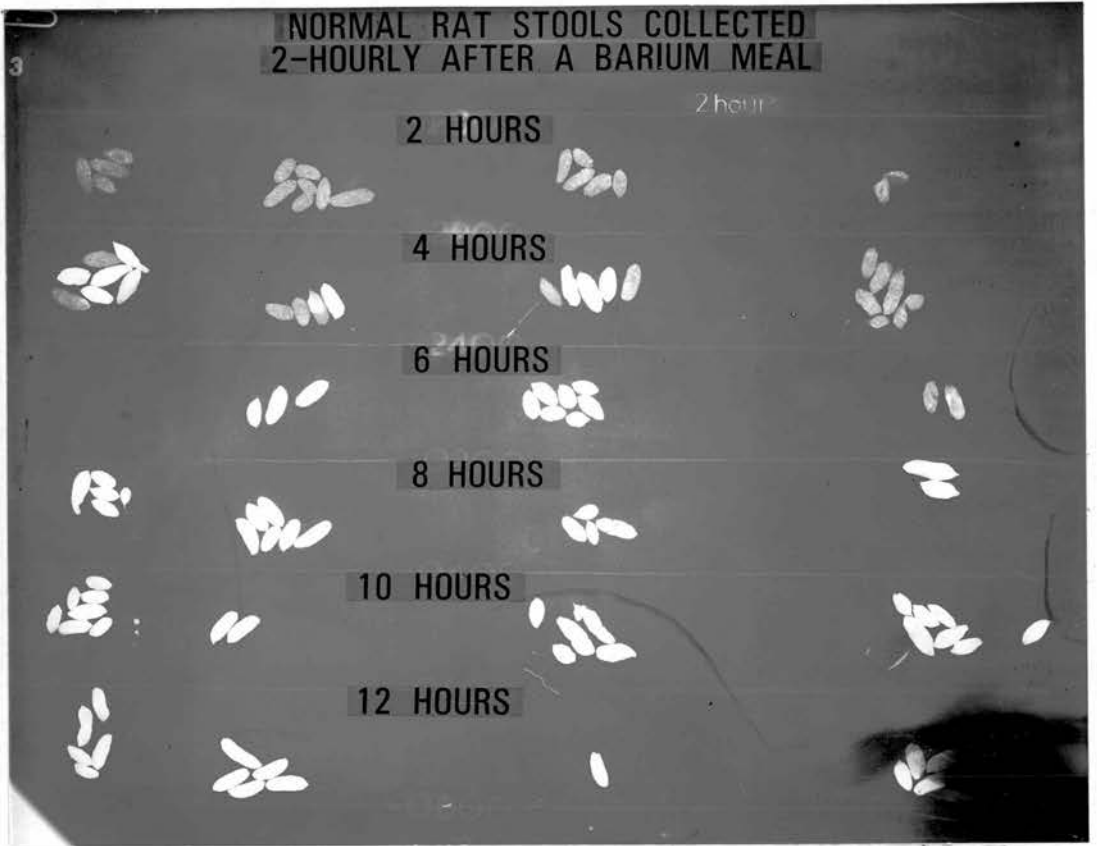


Fig. 9.

Radiograph of normal rat stools. White pellets indicate stools containing barium sulphate.



respectively, the difference being highly significant,  $p < 0.01$ .

Also, the hemicolectomised animals showed a marked delay in stomach emptying time, the mean value being 330 minutes (with a range of 205-475), compared to 185 minutes (range, 145-265 minutes) in controls; the difference is highly significant,  $p < 0.005$ .

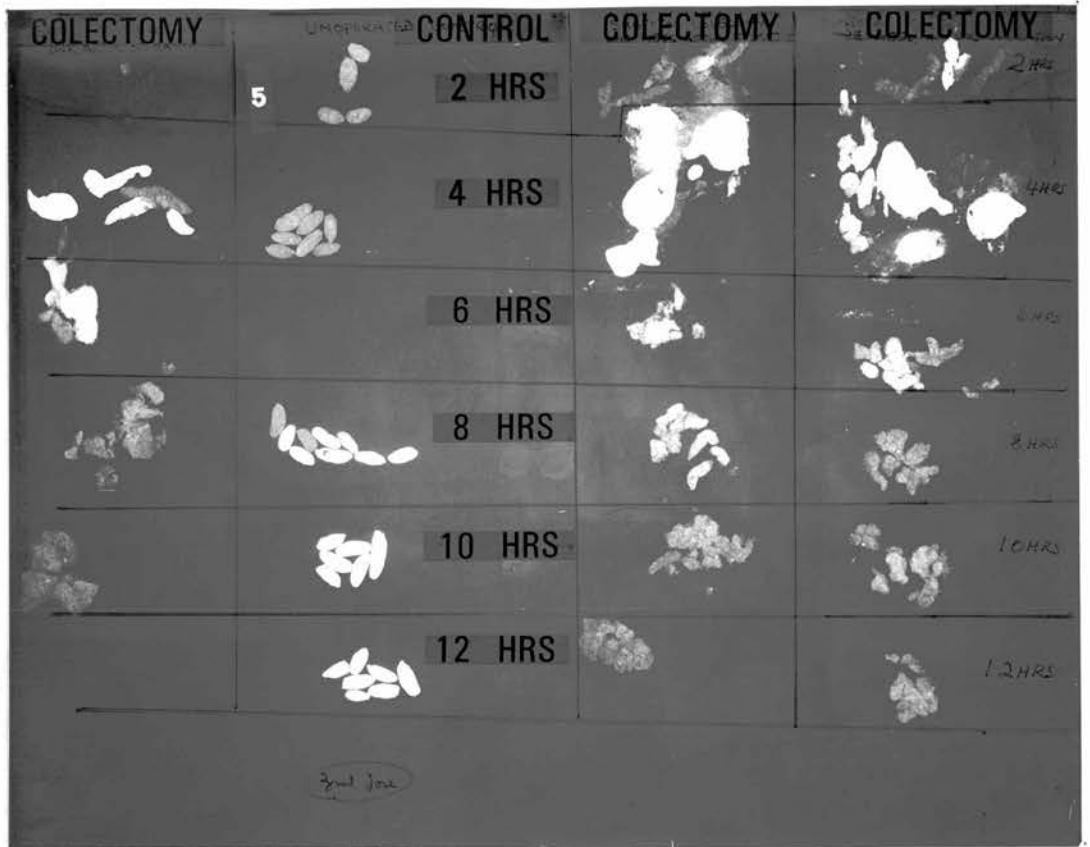
It should be pointed out here that despite the absence of the caecum in the hemicolectomised animals it was not difficult to tell when barium entered the colon: usually in these animals the gut just above and below the anastomosis becomes dilated; therefore, when barium reached this part a spindle-shaped shadow could be seen on screening, indicating entry of barium into the colon. Besides, the colon shadow appeared thicker when compared to the small intestine.

#### (a.ii) Transit-time After Subtotal Colectomy.

The stomach-to-anus transit of a 3ml barium sulphate meal was studied before operation in 12 rats of approximately the same body weight and age. Six of these animals were later subjected to subtotal colectomy, and used for transit time studies at 3 weeks, and again at 3 months after surgery. The remaining six unoperated rats were used as control animals in the two postoperative studies.

Radiological examination of faecal droppings collected at 2-hourly intervals, over 14 hours, showed that 50% of the rats before operation started passing barium-marked stools 6 hours after the introduction of the test meal. All faeces passed 8 to 14 hours after the meal contained barium sulphate (Fig. 9) in all animals.

In contrast, following subtotal colectomy most of the administered barium was evacuated, almost en masse, 4 hours after introduction of the meal (Fig. 10). This was the case both at 3 weeks, and 3 months, after



**Fig. 10.**

A photo-copy of an X-ray picture showing part of the radiograph of stools collected from subtotal colectomised and control rats. Stools were collected at 2-hourly intervals after the test meal, and only those containing barium sulphate appear on the photo-copy.

surgery. Four out of 6 operated rats passed all the meal 8 hours after gavage; the remaining 2 did so at 10 hours. During the postoperative studies two out of 6 controls began passing marked stools at 6 hours; the rest did so 8 hours after the meal; and all the six were still passing barium in their faeces at the end of the study (12 hours after the meal).

---

### Discussion.

Slowing of intestinal transit is one way by which normal bowel function may be restored. Thus, absorption may be facilitated by the prolongation of the duration of contact between mucosal surface and intestinal contents. Whether or not such an adaptive change develops after colectomy in the rat remained to be investigated.

At present there is some evidence that adaptation of gastrointestinal motility, with slowing of transit, occurs at least after partial small bowel resection. Nygaard (1967b) studied intestinal transit in rats (which had previously been subjected to resection of 50% and 75% of the proximal or distal small bowel) by placing a dose, under light anaesthesia, of radioactive chromate into the stomach and then killing the animals at intervals, and ligating the alimentary tract into segments which were then scanned for radioactivity. 50% proximal resection had no significant effect on transit-time; 75% resection significantly reduced the stomach-to-caecum transit-time, as measured 7 days after operation, but during the second postoperative week transit slowed down and later was similar to that in normal animals. There was also a delay in the emptying of the stomach. After distal resections intestinal passage also slowed with time, but transit-time was shorter

than normal even after several months.

Clatworthy et al., (1952) studied the stomach-to-caecum transit-time of barium sulphate by repeated X-ray examination of the gastro-intestinal tract of dogs previously subjected to 50 - 80% enterectomy; although transit (at 7 months after operation) was found to be the same as that in control animals, it was regarded by these workers as delayed, because distance was shortened.

In man, rapid adjustment in gastro-intestinal motility, as judged from the time required for orally administered methylene blue to appear in the stools, has been reported by Althausen et al., (1950), in a patient with only 150cm of distal small bowel remaining; 9 days after operation the dye was passed in  $3\frac{1}{2}$  hours, and three weeks postoperatively, it took 18 hours for the same passage. Similar adaptive changes have been reported by Pilling and Cresson (1957) after studying the stomach-to-anus transit of ingested charcoal, and the stomach-to-caecum passage time of barium sulphate in two infants who had resection of all but 13cm of proximal jejunum and 13 to 15cm of terminal ileum.

In the present study the stomach-to-anus transit of barium sulphate, measured 3 weeks after subtotal colectomy, was greatly shortened, and remained so when measured again three months after the operation. This would seem to indicate inability of the rat gastro-intestinal tract to adapt to the absence of the large bowel by slowing intestinal transit. However, since less than 5cm of terminal large bowel (mainly rectum) remained after subtotal colectomy, the 8 hours required for complete evacuation of the meal would roughly represent the duration of contact between bowel contents and small bowel mucosal surface, which is the same as that observed in rats with right hemicolectomy (*vide infra*).

Following right hemicolectomy the time taken by the head of the barium

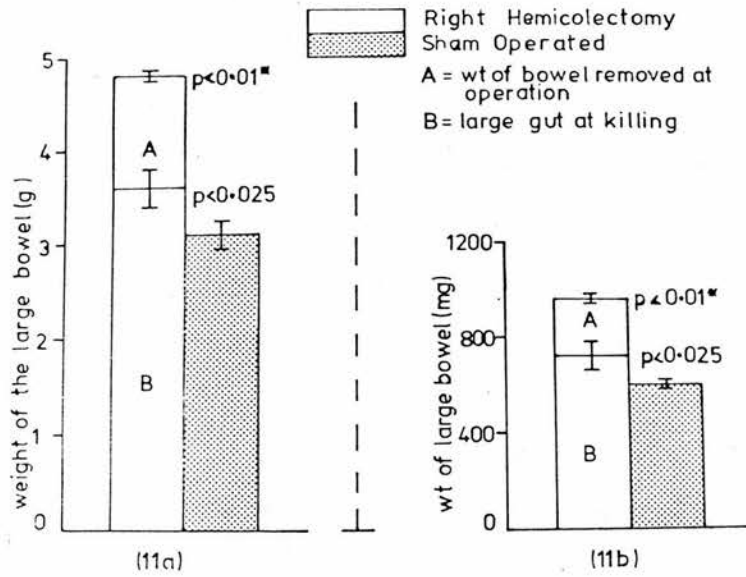
column to traverse the small intestine, measured three months after surgery, was significantly reduced when compared to that in control animals. On the other hand, the small bowel emptying-time was significantly longer in operated than in control animals; the major portion of the test meal appeared to stagnate in the lower part of the small intestine of the hemicolectomised rats. These results indicate that the duration of contact between chyme and mucosal surface, particularly of the lower small intestine, is increased after right hemicolectomy: the digesta from a meal would reach the distal ileum in the hemicolectomised rats about 40 minutes earlier and would remain there for about 1 hour longer than in the control animals. This would give the distal small bowel in operated animals approximately  $1\frac{1}{2}$  hours more of contact between chyme and mucosal surface, which would allow the ileum to absorb more material than normal. In this way some of the nutrients, water and electrolytes not absorbed from the upper small gut (because of intestinal hurry) would be salvaged.

Why the bowel contents after right hemicolectomy appeared to stagnate in the lower small intestine is not known. This could not have been caused by narrowing at the site of anastomosis, because in all animals, at killing, the gut just above and below the anastomosis (about 1cm either side) was thickened and dilated. Narrowing was not seen. It is possible that a localised inco-ordination of muscle contractions (i.e. failure of contractions in the terminal small intestine to synchronise, or couple, with contractions in the bowel below the anastomosis) could result in an inability to propel the bowel contents. This would seem to be supported by the fact that the thickened and dilated gut on either side of the anastomosis was invariably loaded with faecal matter when examined at killing. Hypertrophy and dilatation of

this part of the gut could also be explained on the same basis. On the other hand, the delay in small bowel emptying after right hemicolectomy may partly be accounted for by the prolongation of gastric emptying-time, which was nearly double that in normal controls. What caused the delay in gastric emptying can only be guessed since no attempt was made to investigate the cause. In normal animals, or subjects, control of gastric emptying comes primarily from the duodenum, and depends fundamentally on inhibition of gastric contractions both by nervous (Thomas, Crider and Morgan, 1934) and humoral mechanisms (Farrel and Ivy, 1926). Also, a large number of substances, when present in the duodenum, are known to inhibit gastric emptying; these include acids (Hunt and Knox, 1972), alcohol, hypotonic and hypertonic solutions, amino acids, peptides, sugars and dextrans, fat and fatty acids (Dawson and Engleton, 1968). Included among the humoral factors known to inhibit gastric activity and to slow stomach emptying are: the duodenal hormone - "secretin" - whose secretion is elicited by the presence of acids in the duodenum (Davenport, 1971), and gastrin (Dozois et al., 1970), which is known to stimulate the secretion of gastric acid in the rat (Stanley et al., 1972; Crean et al., 1969) and in the dog (Temperley et al., 1971).

Removal of either the proximal or distal small intestine induces hyperplasia of gastric glands in the rat (Winbon et al., 1974), and causes gastric acid hypersecretion in this animal (Goldman et al., 1972; Ruderman and Kamel, 1970), and in the dog (Hugo et al., 1971). Both the hyperplasia of gastric glands and the hypersecretion of acid can be induced in the intact rat by repeated subcutaneous doses of pentagastrin (Crean et al., 1969; Stanley et al., 1972). The occurrence of high levels of plasma gastrin after partial enterectomy (Straus et al., 1974) would seem to indicate that the gastric acid hypersecretion observed after





**Fig. 11.**

Weight, mean  $\pm$  s.e.m., of the fresh (11a) and dry (11b) large bowel, caecum included, in rats 2 months after right hemicolectomy or sham operation; \* denotes difference between total large bowel (A + B) in colectomised rats and the entire large gut in controls.

partial enterectomy is brought about by gastrin. The demonstration, in subjects with partial small bowel resection, of a fall in the level of plasma gastrin after an initial rise in response to a standard test meal (Straus et al., 1974) is an indication that the high level of plasma gastrin in these subjects is due to hypersecretion, rather than reduced gastrin destruction (Temperley et al., 1971). The cause of this hypersecretion is not known. It is possible that gastrin and gastric acid hypersecretion also occur after colectomy, which would explain the occurrence of a prolonged stomach emptying time.

---

(b) Intestinal Length and Weight.

(b.i) The Colon After Right Hemicolectomy.

WEIGHT.

The weight of the fresh and dry large intestine was studied in 8 right hemicolectomised and 8 sham operated rats 2 months after operation. The mean body weight at killing was  $426 \pm 7$ g and  $452 \pm 6$ g for hemicolectomised and control animals respectively. For each operated rat the weight of the colon and caecum removed at operation was added to that of the colon at killing, and the total was compared to the combined weight of the colon and caecum of the control animal.

The colon and caecum together weighed  $41 \pm 3\%$  more in the hemicolectomy than in control animals (Fig. 11a and 11b). Even when the amount of large intestine removed at operation is neglected, the colon at killing was on average  $14 \pm 3\%$  heavier in operated rats than the combined weight of the colon and caecum in the control animal. In this group the length of the colon was not measured. Rats which were originally SPF were used



Table 8.

Length of colon (caecum excluded), and body weight in rats three months after Right Hemicolectomy (RH) or Sham Operation (SO).

	RH (n=8)	SO (n=8)	paired t-test
Colon removed at operation cm: mean and range	1.6 (1.3 ± 1.8)	nil	
Colon found at killing cm: mean ± s.e.m.	18.9 - 0.7	25.3 - 0.6	p < 0.001
Total colon length cm: mean ± s.e.m.	20.6 - 0.7	25.3 - 0.6	p < 0.005

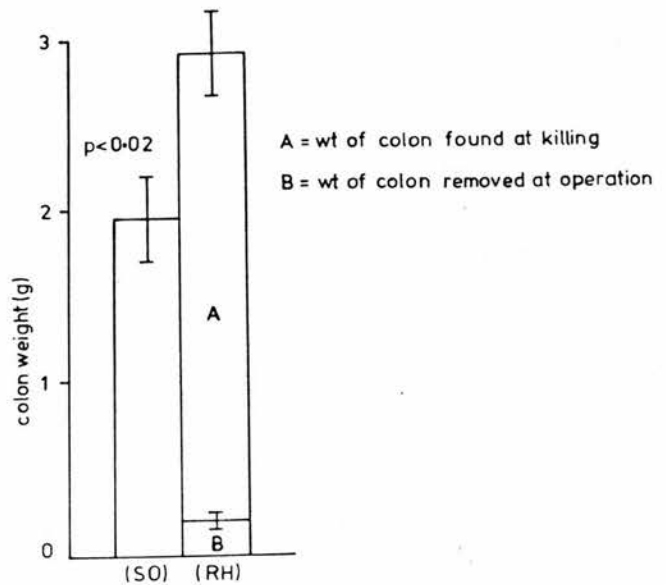


Fig. 12a.

Weight, mean ± s.e.m. of the fresh colon, caecum excluded, three months after right hemicolectomy (RH) or sham operation (SO).

both as operated and control animals.

### LENGTH.

The length of the colon (excluding the caecum) was measured in 8 rats with right hemicolectomy and in 8 sham operated controls three months after surgery. The mean body weight at killing was  $456^{+9}$ g for hemicolectomised rats, and  $470^{+12}$ g for controls. These were locally bred animals. As shown in table 8, three months after operation the colon in hemicolectomised rats was significantly shorter than that in control animals, even when the length of the colon removed at operation was added to that found at killing.

Although the fresh colon at killing was shorter in hemicolectomised rats, it weighed  $37^{+10}\%$  more than the control colon (Fig. 12a), this being true also for the dry colon. When measured per 10cm of gut, the weight of the dry colon at killing was  $83^{+12}\%$  heavier in operated than in control rats, the mean weight being  $240.8 \pm 3.5$ mg and  $136.3 \pm 2.7$ mg for operated and control colon respectively,

The mean dry matter content was approximately the same in operated and control colon. Thus, when the weight of the dry colon was expressed as a percentage of the weight of the fresh gut, the colon in hemicolectomised rats averaged  $17.8^{+1.5}\%$  of dry matter, the corresponding value for sham operated controls being  $17.7^{+0.8}\%$ .

The increase in colon weight was due to increased mucosal and seromuscular mass: the weight of dry mucosa from the colon at killing (excluding the caecum) averaged  $181.8 \text{mg} \pm 7.2$  and  $261.8 \text{mg} \pm 22.3$  in sham operated and hemicolectomised rats respectively, the difference being highly significant,  $p < 0.01$ . The mean seromuscular dry weight for operated and control animals was  $219.5 \text{mg} \pm 20.0$  and  $162.0 \text{mg} \pm 11.8$ , respectively,

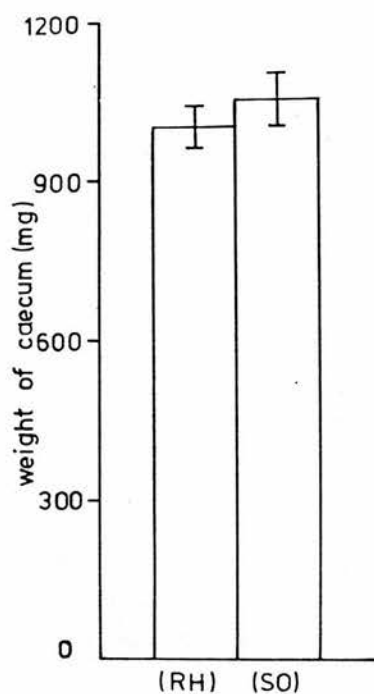


Fig. 12b.

Weight, mean  $\pm$  s.e.m., of the fresh caecum removed at right hemicolectomy (RH) and that found at killing in sham operated controls (SO) three months after surgery.

$p < 0.02$ . Similar differences were observed for the weights of the fresh intestine.

When measured per 10cm of colon the difference in weight of dry mucosa and seromuscular coat was even more striking, the mean mucosal weight being  $130.9\text{mg} \pm 8.2$  and  $72.7\text{mg} \pm 2.8$  for operated and control intestine, respectively,  $p < 0.001$ , while the corresponding values for seromuscular weights were  $109.7\text{mg} \pm 5.1$  for operated and  $64.8\text{mg} \pm 4.7$  for controls,  $p < 0.001$ .

The mucosa/seromuscular (M/S) ratio, calculated from weights of the fresh intestine, was approximately the same in hemicolectomised and control rats, the mean value being  $1.45 \pm 0.1$  and  $1.27 \pm 0.1$  for operated and control intestine, respectively. The increase in seromuscular weight in operated rats, rather than an absence of mucosal enlargement, would seem to account for the similarity between the M/S ratio in operated and control animals.

It was interesting to find that the caecum at killing weighed approximately the same as that removed at operation. At operation both control and operated animals were 3 months of age and weighed nearly the same, the average body weight being  $246 \pm 6\text{g}$  in sham operated controls, and  $247 \pm 4\text{g}$  in hemicolectomised animals. When killed three months later, the control rats weighed on average  $470 \pm 2\text{g}$ , while the hemicolectomised animals averaged  $456 \pm 9\text{g}$ . But in spite of the difference in body weight and age between rats at killing and rats at operation there was no significant difference between the caecum removed at operation and that at killing, (Fig. 12b). This is an indication that during the period between surgery and killing the caecum grew little or not at all. Indeed, the weight of the large intestine as a whole, as studied in normal rats (sham operated and unoperated) weighing between 250 and 549g (body weight range for all animals used for gut weight studies) showed little change, (Fig. 13).

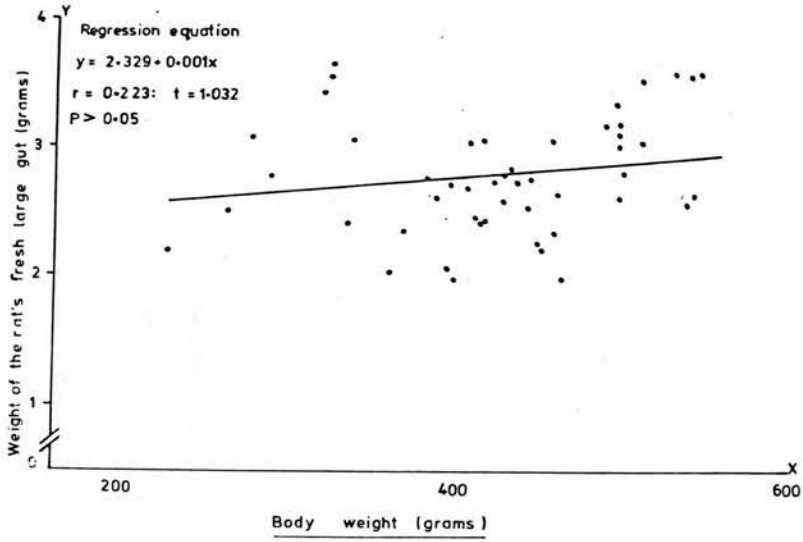


Fig. 13.

Relationship between the weight of the large intestine and body weight in rats weighing between 250 - 579 grams. Each dot represents one rat.

Table 9.

Length of Colon (caecum excluded) in rats three months after Left Hemicolecotomy (LH) or Sham Operation (SO)

	<u>LH (n=8)</u>	<u>SO (n=8)</u>	<u>paired t-test</u>
Colon removed at operation cm: mean and range.	3.7 (2.8 - 5.0)	nil	
Colon found at killing cm: mean $\pm$ s.e.m.	15.3 $\pm$ 0.4	23.4 $\pm$ 0.4	p < 0.001
Total length of colon cm: mean $\pm$ s.e.m.	18.4 $\pm$ 0.3	23.4 $\pm$ 0.4	p < 0.001

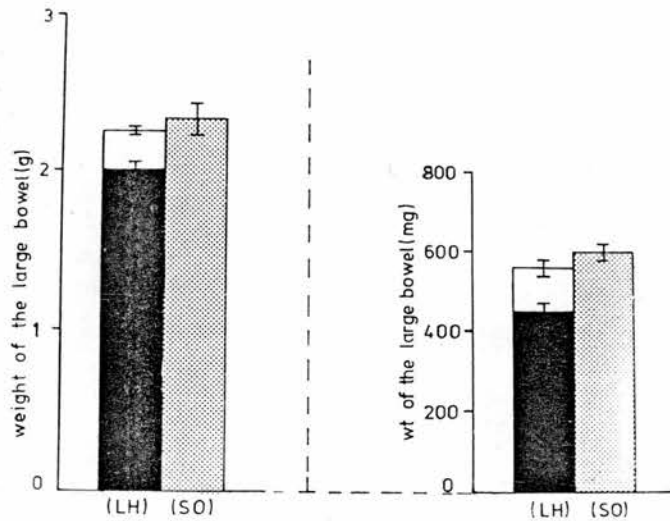


Fig. 14.

Weight, mean  $\pm$  s.e.m., of the fresh (left) and dry (right) large intestine, caecum included, in rats with left hemicolecotomy (LH) and in sham operated (SO) controls. Shaded area denotes the weight of large gut found at killing; the unshaded area representing colon removed at operation.

(b.ii) The Colon After Left Hemicolectomy.

Length.

Animals were killed three months after operation, and weighed approximately the same, the mean body weight for hemicolectomised and control rats being  $364^{+10}g$  and  $376^{+14}g$ , respectively. The length of the colon (excluding the caecum) at killing was  $39^{+3}\%$  shorter in rats with distal colon resection than in sham operated controls, even when the amount of colon removed at operation was added to that found at killing (Table 9.), the total colon length was still  $22^{+4}\%$  shorter in operated than in control rats.

Colon Weight.

The wet and dry weight of the colon (including the caecum) at killing was significantly heavier in the control than in the hemicolectomised rats,  $p < 0.02$ ; but when the amount of colon removed at operation was added to that found at killing there was no significant difference between the total colon weight in operated and in control rats (Fig. 14.)

However, since the colon was shorter in the operated than in control animals, to ascertain whether after left hemicolectomy the colon increased in mass, it was necessary to measure weight per unit length of colon. As shown in Fig. 15, when the weight of the colon at killing was measured per 10cm, the colon in operated rats was on average  $33^{+4}\%$  heavier than in sham operated controls.

On the other hand, there was no significant difference between the weight of the caecum in rats with left hemicolectomy and in sham operated controls, the mean values for the fresh caecum being  $875.5^{+23.0}mg$  in operated, and  $930.0-45.8mg$  in control animals. The dried organ weighed approximately the same in the two animal groups. Also, the content of

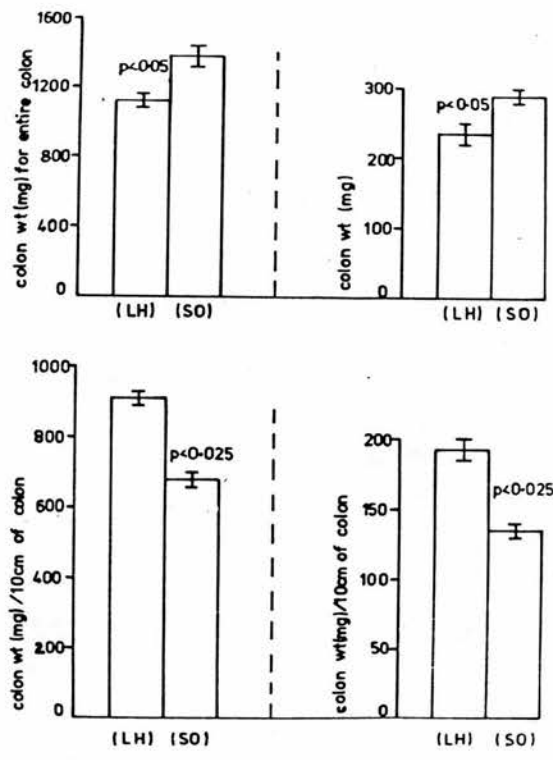
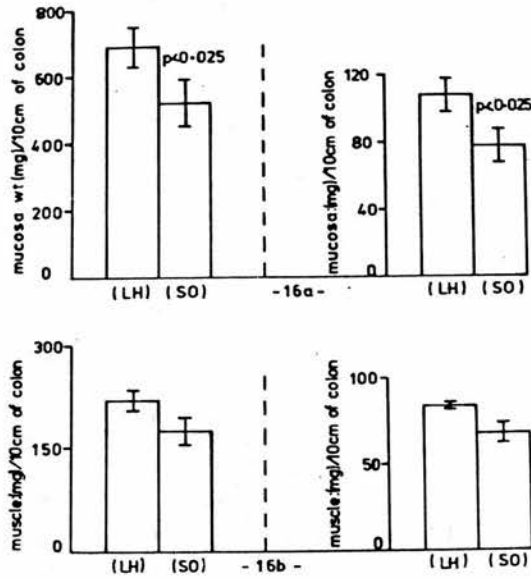


Fig. 15. Weight, mean  $\pm$  s.e.m., of the fresh (left) and dry (right) colon, excluding caecum, at killing in rats with left hemicolectomy (LH) or sham operation (SO) as measured for whole colon - above - and per 10cm, below.





Figs. 16a and 16b.

Weight, mean  $\pm$  s.e.m., of the fresh (left) and dry (right) mucosa "16a" and seromuscular coat "16b" of the colon at killing in rats with distal hemicolecotomy (LH) or sham operation (SO).

dry matter in the colon (for mucosa and seromuscular coat together) was approximately the same in operated and in control rats, the average being  $17.8\% \pm 0.7$  and  $16.5\% \pm 1.4$  for operated and control animals, respectively.

By weighing mucosa and seromuscular coat separately it was found that the increase in colon weight was mainly confined to the mucosa (Fig. 16a). Thus, whereas the seromuscular coat showed no significant increase (Fig. 16b) mucosal weight increased by  $24\% \pm 6\%$ . The percentage of dry matter both in mucosa and the seromuscular coat was not significantly different in the two groups, the mean mucosal dry matter content being  $15.7\% \pm 0.7$  in operated rats, and  $14.9\% \pm 1.2$  in controls. The corresponding values for the seromuscular coat were  $18.9\% \pm 0.6$  and  $18.7\% \pm 3$  for hemicolectomy and control animals, respectively.

Although mucosal mass, measured per 10cm of colon, was definitely increased after left hemicolectomy the M/S ratio was approximately the same in operated and control colon. Operated rats had a mean M/S ratio of  $1.65 \pm 0.16$ , compared to  $1.32 \pm 0.12$  in control animals. The relative increase in the seromuscular mass in the operated colon (Fig. 16b) may explain the similarity between the colon M/S ratio observed in operated and control animals.

---

### Discussion.

When measured in mg/10cm, the weight of the remnant of colon increased more after right hemicolectomy ( $83\% \pm 12\%$  above control value) than after distal resection ( $33\% \pm 3\%$  greater than normal). This increase in weight, interpreted as growth, involved both the mucosa and the seromuscular coat in proximal resection, but was mainly confined to the

mucosa in distal colectomy. In both right and left resections the remnant of colon did not grow in length.

The colon thus joins the list of organs which show compensatory hypertrophy after partial excision, especially when the excision is of proximal colon. Compensatory hypertrophy is well known in the liver (Fishback, 1929; Bucher and Swaffield, 1964; Bucher, 1967). Following partial hepatectomy the lobes in the remaining part of the liver undergo a hyperplastic budding process at their periphery, with production of new lobes similar in size and shape to the old ones. In this organ the degree of regenerative response seems to be related to the size of partial hepatectomy (MacDonald et al., 1962) and, in the rat, is more marked in young than in old animals (Bucher et al., 1967; Bucher and Swaffield, 1964). Compensatory hyperplasia also occurs in the remaining kidney in some cases after unilateral nephrectomy (Addis et al., 1927; Hayslett, 1968; Lytton et al., 1969; Potter et al., 1969).

What caused the colon to enlarge was not investigated; however, an inference can be made concerning the most likely factors to be involved. Previous studies have demonstrated that the colon in bulk-fed rats shows marked increase in length, mucosal surface area (Weirda, 1942) and weight (Weirda, 1950; Moinuddin and Lee, 1959; Fischer, 1959; Dowling et al., 1967) when compared to that in rats of similar body weights and age, which have been maintained on a normal diet. Bulk-feeding induces hyperphagia (Addis, 1932; Adolf, 1947; Moinuddin and Lee, 1959) which may reach three to four times normal intake (Weirda, 1950; Dowling et al., 1967), depending on the degree of diet dilution. This hyperphagia is considered by Addis (1932) to be partly responsible for the bowel enlargement.

During lactation the rat large intestine increases in length and

weight (Cripps and Williams, 1975); there is marked hypertrophy of the caecum, small intestine and the stomach (Fell et al., 1963), and food consumption increases to two to three times normal intake (Cole and Hart, 1938; Anderson and Turner, 1963; Fell et al., 1963). Restricting the food intake of lactating rats seems to abolish intestinal hypertrophy, except of the caecum (Campbell and Fell, 1964), indicating that increased food intake is partly responsible for the enlargement of gut during lactation.

Wilmore et al. (1971) observed marked hypertrophy of the colon and caecum in 13 dogs one year after massive small bowel resection; this enlargement was associated with hyperphagia amounting to 3 - 6 times the normal intake.

The influence of increased topical nutrition on mucosal enlargement is well established for the small intestine (Dowling and Booth, 1967, Gleeson et al., 1972a; Dowling, 1967), but less well documented for the colon. Starvation in the rat (for 6 days) decreases villus size, and reduces mitosis in the crypts; refeeding restores both villus size and mitosis (Altmann, 1972).

The growth pattern adapted by the rat large intestine after exposure to increased food intake seems to be determined by the composition of the diet ingested. When hyperphagia is associated with a normal rather than a diluted diet, e.g. during lactation, the net nutrient intake increases; bowel enlargement then seems to involve both the mucosa and the muscle coat (Fell et al., 1963). On the other hand, when the unabsorbable bulk in the diet is increased, the net nutrient intake may be normal or reduced in spite of hyperphagia; in such a situation the weight increase in the colon seems to be principally in the seromuscular coat (Dowling et al., 1967).

In the present study, following right hemicolectomy the mucosa of the remnant colon increased by  $44^{+1}\%$ , and the seromuscular coat by  $36^{+3}\%$ , which would seem to indicate that the colon was exposed to increased nutrition and, probably, a high dietary bulk. Judging from the stomach-to-colon transit of barium sulphate it would appear that right hemicolectomy hastens the transit of chyme from the small intestine into the colon. In the presence of a shortened intestinal transit, less absorption would take place in the upper intestine (Hammer *et al.*, 1959); consequently, the chyme bathing the colon would contain more nutrients than normal. Mucosal hypertrophy may thus develop as a result of increased nutrition even though hyperphagia does not occur.

The occurrence of increased faecal dry matter output seems to indicate that after removal of the caecum, much dietary cellulose enters the colon undigested, as if the diet contained a high residual content. Since there was no evidence to indicate chronic intestinal obstruction (which may cause gut muscle hypertrophy), a high residual bulk is the most likely cause of increased seromuscular weight in the remnant colon after proximal resection. The occurrence of decreased digestibility of the rat diet following caeectomy (Demarne *et al.*, 1973) would seem to support this suggestion, although Fischer (1959) failed to demonstrate a relationship between increased amounts of faecal residue and the weight or size of the rat large intestine (caecum in particular).

Although the enlargement of the colon after right hemicolectomy seems to fit the "increased intraluminal nutrition hypothesis", that after left colectomy cannot be explained on this basis. Other factors are likely to be involved, and are discussed in the following section.

---

Table 10.

Body weight and total length of the small intestine of male albino rats: measurements of length being made on fresh intestine taken from right hemicolectomised (RH), sham operated (SO) and unoperated (UC) rats.

Body weight range (g).	Animal group.	Number of Animals.	Mean Body Weight (g) $\pm$ s.e.m.	Mean intestinal length (cm) $\pm$ s.e.m.
350 - 399	RH	5	361 $\pm$ 8	127.2 $\pm$ 0.9
	SO	5	365 $\pm$ 9	126.4 $\pm$ 1.7
	UC	6	367 $\pm$ 9	128.0 $\pm$ 3.7
400 - 449	RH	11	424 $\pm$ 6	128.6 $\pm$ 2.8
	SO	10	426 $\pm$ 5	125.6 $\pm$ 1.7
	UC	8	426 $\pm$ 6	130.2 $\pm$ 2.4
450 - 499	RH	5	472 $\pm$ 10	136.3 $\pm$ 4.2
	SO	8	480 $\pm$ 6	139.4 $\pm$ 2.3
	UC	5	470 $\pm$ 3	136.2 $\pm$ 1.7
500 - 549	RH	7	517 $\pm$ 6	138.8 $\pm$ 2.2
	SO	8	525 $\pm$ 6	138.5 $\pm$ 2.3
	UC	6	513 $\pm$ 7	136.6 $\pm$ 2.1
550 - 599	RH	2	599.0	145.0 -
	SO	1	574.5	142.0 -
	UC	-		
All groups (350 - 599)	RH	30	458 $\pm$ 12	133.4 $\pm$ 2.2
	SO	32	465 $\pm$ 11	132.1 $\pm$ 1.5
	UC	24	452 $\pm$ 10	132.3 $\pm$ 1.4

(b.iii) The Small Intestine After Right Hemicolectomy.

Length.


The length of the small bowel in operated and control animals was approximately the same. To obtain results which are representative of a large number of observations measurement of small bowel length was made on fresh intestine taken from 86 rats. These included 30 right hemicolectomy, 32 sham operated and 24 unoperated rats, all belonging to various groups of animals used in this work. As shown in table 10, for animals of comparable body weights, the length of small intestine was not significantly different in colectomised, sham operated and unoperated rats. On the other hand, in all the three groups, the small intestine grew longer with increasing body weight.

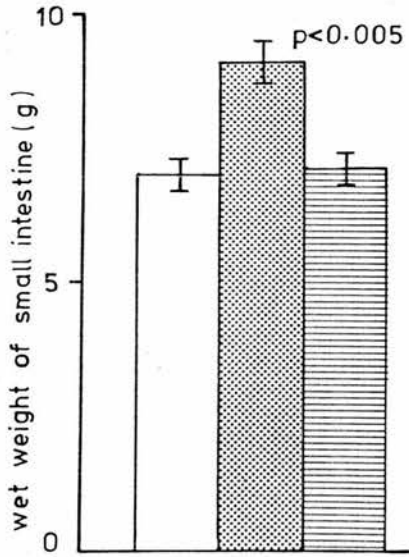
Small bowel weight.

The small intestine both fresh and dry was 20 to 25% heavier in rats with right hemicolectomy than in either sham operated or unoperated controls (Fig. 17a and 17b). On the other hand there was no significant difference between the small bowel weight in sham operated and unoperated control groups.

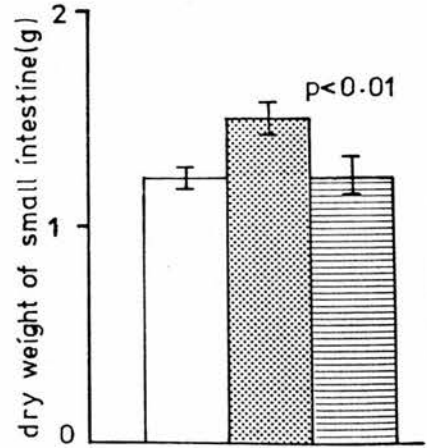
The mean dry matter content in the small bowel was about the same in operated and in control rats. Thus, when dry bowel weight was expressed as a percentage of the wet weight, the small intestine in hemicolectomised, sham operated and unoperated rats averaged  $16.7\% \pm 1.5$ ,  $16.7\% \pm 0.6$  and  $16.0\% \pm 0.4$  of dry matter, respectively.

Although the entire length of small bowel in right hemicolectomised rats increased in weight (Fig. 18), the major change occurred in the lower third, where the location of weight change was principally the mucosa and not the seromuscular coat (Figs. 19a and 19b). The difference was the same even when mucosa was measured per 10cm of


 Unoperated controls  
 Right hemicolectomy  
 Sham operated controls



(17a)



(17b)

Fig. 17.

Weight, mean  $\pm$  s.e.m., of the fresh (17a) and dry (17b) small bowel in right hemicolectomised, sham operated and unoperated rats.



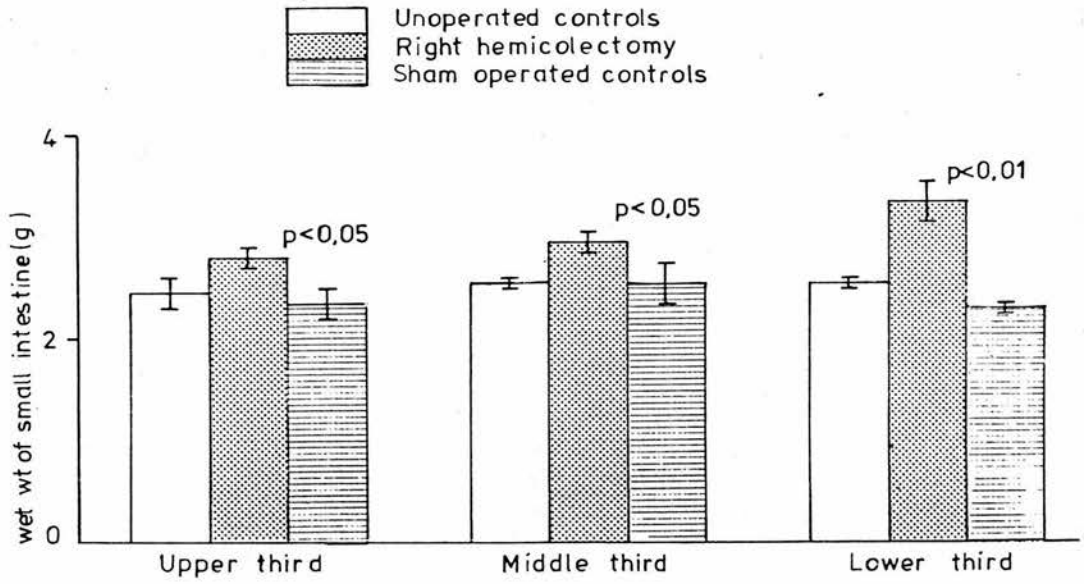


Fig. 18.

Weight, mean  $\pm$  s.e.m., of the upper, middle and lower thirds of the small gut in rats with right hemicolectomy, sham operation and in unoperated controls.

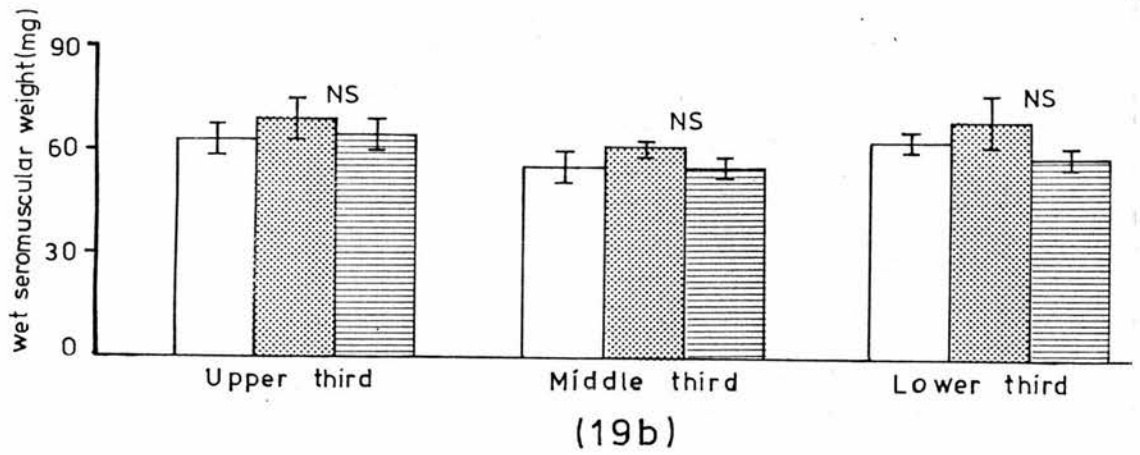
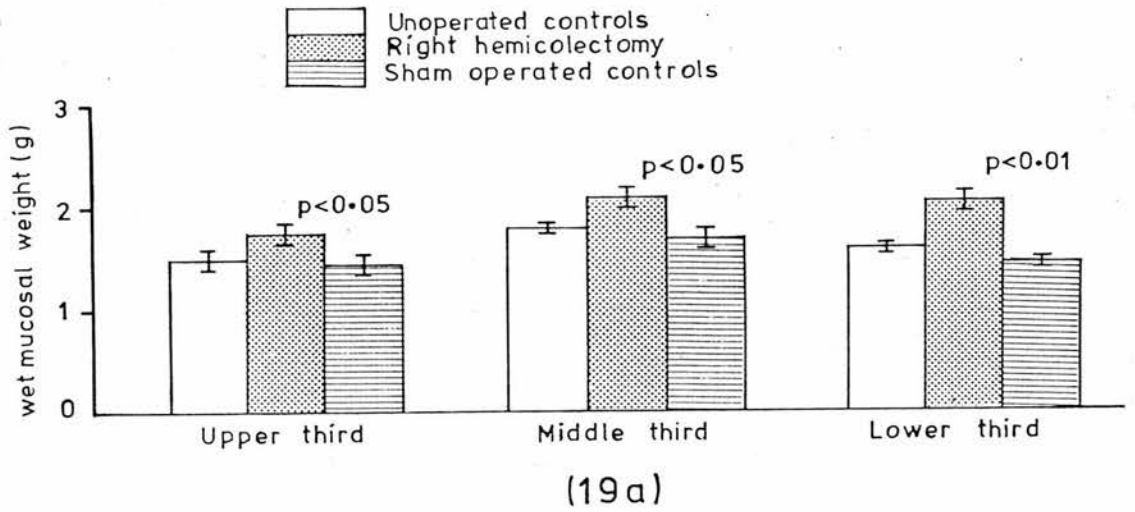


Fig. 19.

Average weight ( $\pm$  s.e.m.) of mucosa (above) and seromuscular coat (below) from the upper, middle and lower thirds of the small intestine in right hemicolecotomised, sham operated and unoperated rats.

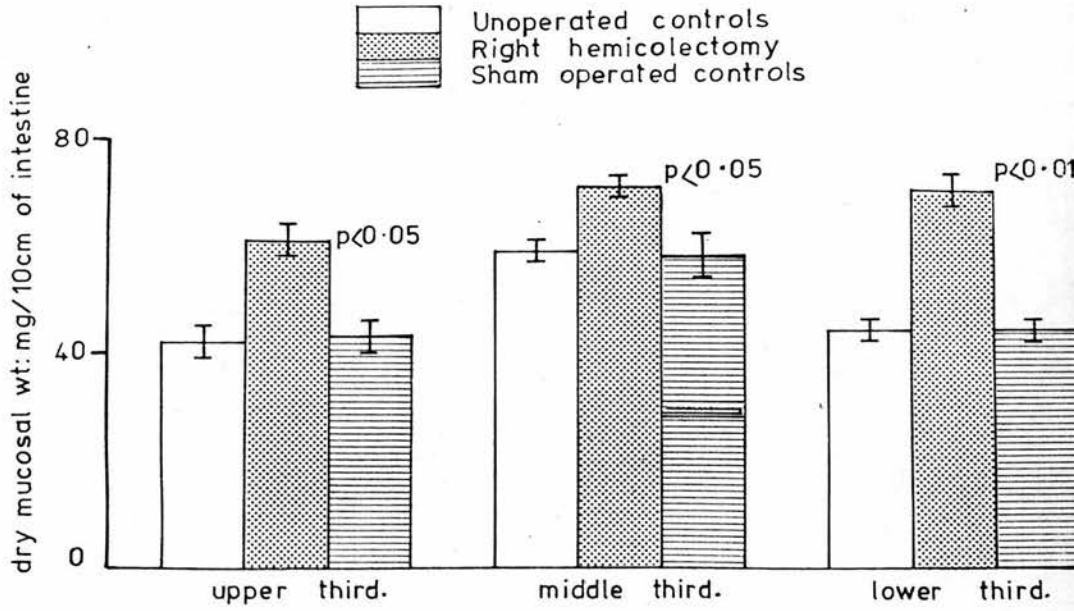


Fig. 20.

Mucosal weight (mean  $\pm$  s.e.m.) per 10cm of small intestine, measured for upper, middle and lower thirds.

intestine (Fig. 20).

Mucosa/Seromuscular (M/S) ratios for the three segments were calculated from wet weights; when compared (right colectomy vs sham operated, and right colectomy vs unoperated, according to body weight) no significant difference was observed between operated and control animals (Table 11.). On the other hand, in all animal groups the M/S ratio for the middle third of the small intestine was significantly greater than either that of upper or lower third.

Since mucosal weight, particularly of the lower third, was definitely increased after right hemicolectomy, one would expect a similar increase in the M/S ratio. This, however, is true only if the seromuscular coat does not increase in mass. It is probable that the relative increase in the seromuscular weight in this case (Fig. 19b), though not statistically significant, may have reduced the M/S ratio in colectomy bowel. In the present study, therefore, the absence of increased M/S ratio in hemicolectomy rats does not indicate lack of mucosal enlargement.

(b.iv) Small Bowel Length and Weight After Left Hemicolectomy.

The small intestine in operated and control animals was approximately the same in length, the mean values being  $118.0\text{cm} \pm 3.0$  and  $120.0\text{cm} \pm 4.7$ , for operated and control rats, respectively. The small bowel after distal colon resection did not increase in mucosal or seromuscular mass (Table 12), though it did after right hemicolectomy.

(b.v) Length and Weight of the Small Intestine After Subtotal Colectomy.

Length.

8 rats with subtotal colectomy and 8 sham operated controls were

Table 11.

Mucosa / Seromuscular (M/S) ratio in the small intestine of right hemicolectomy (RH), sham operated (SO) and unoperated (UC) rats studied three months after operation.

Intestinal segment.	RH (n = 8)	SO (n = 8)	UC (n = 8)
Upper Third mean M/S ratio <sup>±</sup> s.e.m.	1.66 ± 0.07	1.65 ± 0.08	1.94 ± 0.17
Middle Third mean M/S ratio <sup>±</sup> s.e.m.	2.64 ± 0.14*	2.25 ± 0.13*	2.51 ± 0.14*
Lower Third mean M/S ratio <sup>±</sup> s.e.m.	1.73 ± 0.12	1.57 ± 0.08	1.68 ± 0.16

\* In all three groups the middle third M/S ratio was significantly greater than either the M/S ratio for upper or lower third,  $p < 0.01$  in each case (paired 't' test).

Table 12

Wet and dry weights of mucosa and the seromuscular coat of the small intestine in rats with left hemicolectomy (LH) and in sham operated (SO) controls.

segment	WET WEIGHT		DRY WEIGHT		
	LH (n=8)	SO (n=8)	LH (n=8)	SO (n=8)	
<u>UPPER</u>	Mucosa,mg/10cm of intestine: mean $\pm$ s.e.m.	556.2 $\pm$ 50.3	595.5 $\pm$ 45.9	74.4 $\pm$ 5.9	77.6 $\pm$ 7.8
	Muscle,mg/10cm of intestine: mean $\pm$ s.e.m.	129.7 $\pm$ 7.9	128.3 $\pm$ 3.4	22.6 $\pm$ 2.4	21.9 $\pm$ 2.3
<u>MIDDLE</u>	Mucosa,mg/10cm of intestine: mean $\pm$ s.e.m.	573.7 $\pm$ 20.9	608.7 $\pm$ 50.7	75.7 $\pm$ 4.5	79.9 $\pm$ 5.6
	Muscle,mg/10cm of intestine: mean $\pm$ s.e.m.	128.8 $\pm$ 15.6	129.3 $\pm$ 16.4	21.5 $\pm$ 2.2	21.2 $\pm$ 1.9
<u>LOWER</u>	Mucosa,mg/10cm of intestine: mean $\pm$ s.e.m.	543.5 $\pm$ 30.8	511.5 $\pm$ 46.1	71.9 $\pm$ 2.5	68.7 $\pm$ 5.6
	Muscle,mg/10cm of intestine: mean $\pm$ s.e.m.	121.7 $\pm$ 4.6	118.0 $\pm$ 8.2	20.9 $\pm$ 1.4	19.8 $\pm$ 1.5

Following resection of the distal colon the small intestine does not increase in weight.

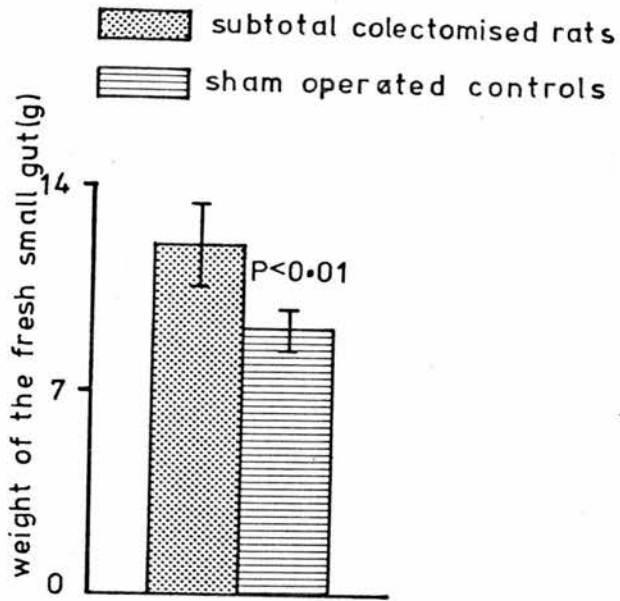


Fig. 21.

Weight of the fresh small intestine in rats, 4 months after subtotal colectomy, or sham operation. Values represent the mean and s.e.m.

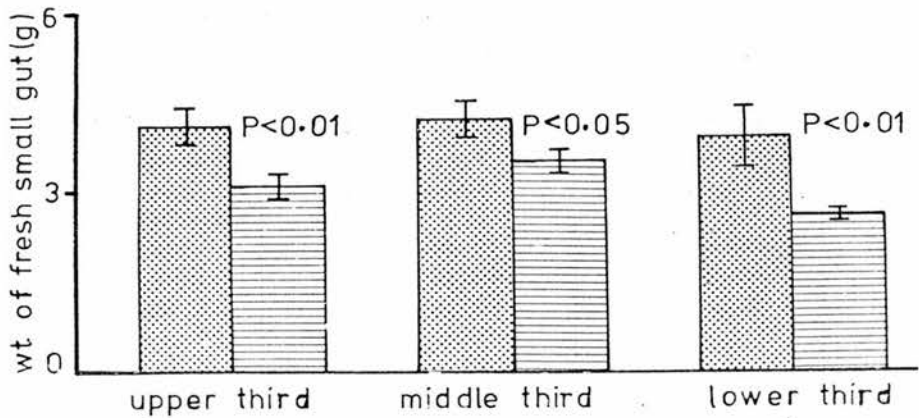


Fig. 22.

Location of weight increase in the rat small intestine, 4 months after subtotal colectomy (dotted blocks), as compared to sham operated intestine (line stippled blocks). Values represent the mean and s.e.m.

killed 8 months after operation. At killing operated and control animals were of the same age and weighed approximately the same, mean body weights for operated and control animals being  $519.6g \pm 18.7$  and  $523.4g \pm 16.4$ , respectively. Small bowel length averaged  $137.9cm \pm 1.6$  in colectomised rats, and  $139.2cm \pm 1.9$  in controls, the difference being not significant.

#### Weight.

The small intestine in operated rats weighed  $33 \pm 0.7\%$  more than in control animals (Fig. 21). The increase in weight was more marked in the upper and lower thirds of the small intestine (Fig. 22), and was particularly confined to the mucosa (Fig. 23a), although the sero-muscular coat of the lower third was also significantly heavier in operated than in control animals (Fig. 23b). Unoperated controls were not used because no difference in gut weight was demonstrated between these animals and sham operated controls.

#### (b.vi) Small Bowel Length and Weight After Subtotal Colon Bypass.

8 rats with subtotal colon bypass were compared to 8 subtotal colectomies, three months after operation. The mean length of the small intestine was about the same in the two groups: it averaged  $128.0cm \pm 2.6$ , with a range of 108 - 142cm, in the colon bypass group; and  $126.9cm \pm 1.3$ , and a range of 99 - 143cm, in subtotal colectomies.

#### Weight.

The weight of the small bowel, measured for the entire length, and for upper, middle and lower thirds, was approximately the same after colon bypass and after subtotal colectomy (Table 13).



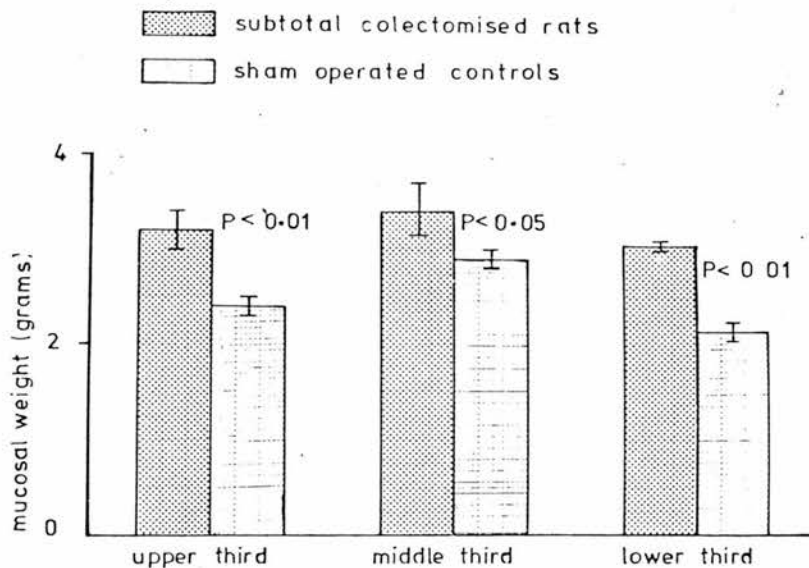


Fig. 23a.

Weight of fresh small gut mucosa (mean  $\pm$  s.e.m.) four months after subtotal colectomy or sham operation. Differences remain unchanged when weights are expressed per 10cm of small intestine.

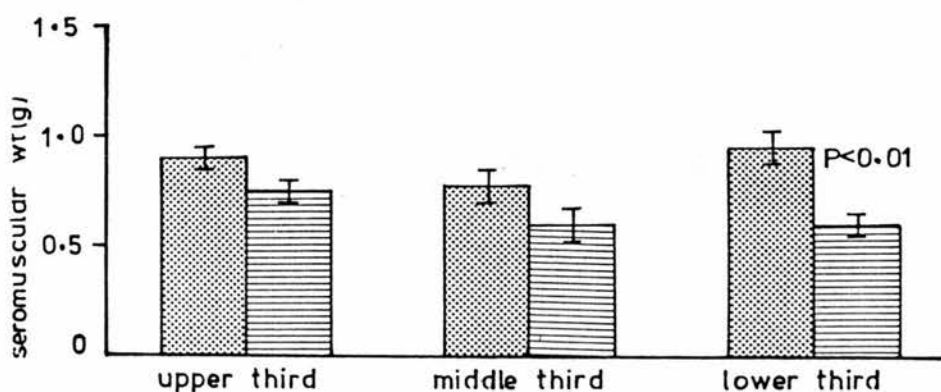


Fig. 23b.

Weight of fresh small gut seromuscular coat (mean  $\pm$  s.e.m.) four months after subtotal colectomy or sham operation. Differences remain the same when weights are expressed per 10cm of gut.

Table 13.

Weight of the fresh small intestine in rats with Subtotal Colectomy (SC) or Colon Bypass (CB), three months after surgery.

specimen.	animal group.	Intestinal Segment.		
		Upper	Middle	Lower
whole segment (mucosa and muscle together) (g): mean $\pm$ s.e.m.	SC	3.10 $\pm$ 0.21	3.37 $\pm$ 0.23	3.04 $\pm$ 0.44
	CB	2.96 $\pm$ 0.26	3.31 $\pm$ 0.25	2.98 $\pm$ 0.17
mucosa alone, from whole segment, (g): mean $\pm$ s.e.m.	SC	2.54 $\pm$ 0.13	2.83 $\pm$ 0.23	2.44 $\pm$ 0.15
	CB	2.37 $\pm$ 0.11	2.79 $\pm$ 0.13	2.39 $\pm$ 0.26
Seromuscular coat alone; from whole segment. (mg): mean $\pm$ s.e.m.	SC	642.7 $\pm$ 66.16	543.9 $\pm$ 97.82	597.5 $\pm$ 42.85
	CB	579.3 $\pm$ 41.27	529.2 $\pm$ 62.29	589.1 $\pm$ 42.22
mucosa and seromuscular coat together, from the entire small intestine. (g): mean $\pm$ s.e.m.	SC	9.47 $\pm$ 0.42		
	CB	9.09 $\pm$ 0.25		

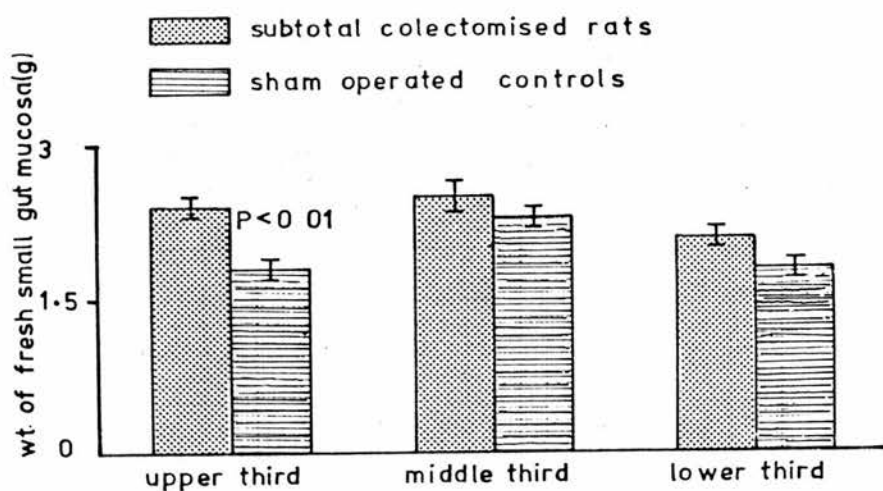


Fig. 24a.

Weight of small gut mucosa (mean  $\pm$  s.e.m.) after three weeks of pair feeding, in rats subjected to subtotal colectomy or sham operation three months previously.

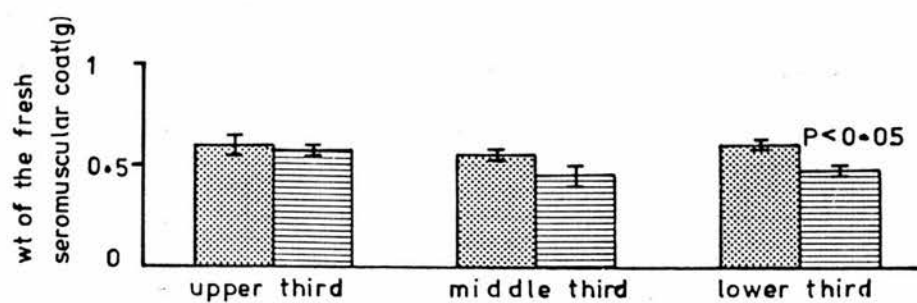


Fig. 24b.

Weight of the seromuscular coat of the small gut (mean  $\pm$  s.e.m.) after three weeks of pair feeding, in rats subjected to subtotal colectomy or sham operation three months previously.

(b.vii) The Small Intestine in Subtotal Colectomised rats  
after Pair Feeding.

At the end of three weeks of pair feeding, 8 subtotal colectomised and 8 sham operated rats, weighing on average  $407.4 \pm 3.2$ g and  $464.5 \pm 3.9$ g, respectively, were killed. The small intestine averaged  $127.6 \pm 3.4$ cm in operated animals, and  $125.6 \pm 2.7$ cm in controls; and weighed  $8.8 \pm 0.3$ g and  $7.6 \pm 0.2$ g in operated and control animals, respectively, being  $16.9 \pm 0.4\%$  heavier in the subtotal colectomy group. The location of weight change was mainly the mucosa of the upper third (Fig. 24a), and the seromuscular coat of the lower third of the intestine (Fig. 24b).

---

Discussion.

Resection of the distal colon caused no change in small bowel weight, whereas right hemicolectomy induced a 23% increase in small gut weight. Enlargement was mainly confined to the mucosa, particularly of the lower third of the intestine, and occurred in the absence of hyperphagia. On the other hand, subtotal colectomy induced a slightly more marked increase in small bowel weight (33% above control value), which involved mostly the mucosa of the upper and lower thirds of the small intestine, although the seromuscular coat of the distal third was also significantly increased; food intake was increased by 30 to 40%. The hyperphagic subtotal colectomised rat had a small gut 33% heavier than control bowel, but after three weeks of pair feeding the small bowel in colectomised animals was only 16% above control value. It would appear, therefore, that the hyperphagia accounted for about 50% of the small bowel weight increase. Subtotal colon bypass had similar effect on small bowel weight as subtotal colectomy.

A number of hypotheses relating to the mechanism by which growth of gut remnant may be induced have been put forward. These include:-

(a) The Exogenous Intraluminal Stimulation Hypothesis, which explains, for example, why the ileum will hypertrophy when inserted into the jejunum; and the jejunum atrophy when transposed to the ileum (Altmann, 1971; Altmann and Leblond, 1970), and also explains why compensatory enlargement in the small bowel is more marked in ileal than in jejunal remnants (Nygaard, 1967a; Dowling and Booth, 1967b).

Included in this hypothesis are two theories: the first proposes the existence of villus enlarging and reducing factors in the intestinal chyme (Altmann and Leblond, 1970). Thus, villus enlarging factors have been shown to exist in the secretions of the rat stomach, duodenum and pancreas (Altmann and Beblond, 1970; Altmann, 1971) and are thought to be responsible for the occurrence of tall villi in the upper intestine (Altmann and Leblond, 1970). It would seem that these factors exert their villus enlarging effect by causing increased epithelial protein synthesis (Altmann, 1974). The villus reducing factors have been demonstrated in the rat ileum; these appear to be products of stagnant bile and bile acids (Altmann, 1974).

The second theory postulates that nutritional elements in the diet, which are normally absorbed proximally, regulate mucosal growth and absorptive capacity in the small intestine; thus, after resection or bypass of the upper part, the chyme reaching the distal small intestine contains more nutrients than normal, and induces mucosal hypertrophy to develop as a result of the high nutrient content (Dowling and Booth, 1967; Dowling, 1967). This hypothesis is supported by a good deal of experimental findings, all of which seem to indicate that increased

alimentation resulting from hyperphagia (associated with a normal rather than a diluted diet) stimulates structural and functional changes in the gastrointestinal tract. For example, during pregnancy and lactation in the rat, increases occur in the small gut length (Pool, Lew and Addis, 1939; Souders and Morgan, 1957; Craft, 1970), mucosal surface area (Craft, 1970; Fell, 1972), some mucosal enzymes (Rolls, 1975) and in the absorptive capacity e.g. for glucose and L-leucine (Cripps and Williams, 1975; Cripps, 1972), although in vivo absorption studies, using a closed loop method, showed decreased glucose and glycine absorption per unit mucosal area in the jejunum of pregnant rats (Craft, 1970). It has been pointed out before that food intake in the lactating rat is more than doubled, and the intestinal changes are attributed to the hyperphagia by Campbell and Fell (1964), although Elias and Dowling (1974) reported that ... "exclusion of pancreatic secretions from the jejunum and complete exclusion of exogenous luminal nutrition and duodenal secretions do not further modify the structural changes seen in jejunum during lactation ..... Similarly, diversion to the ileum of greater than normal amounts of bile and pancreatic secretions, or of luminal contents directly from the stomach and duodenum do not further significantly increase villus height or crypt depth"; indicating that hormonal changes may also play a part in lactational gut hypertrophy.

Intestinal enlargement, with mucosal hypertrophy, has also been observed in cold adapted rats (Heroux and Gridgeman, 1959), in rats adapted to intermittent starvation (Holeckova and Fabry, 1959; Fabry and Kujalova, 1960), following hypothalamic damage (Brobeck, Tepperman and Lang, 1943) and in the alloxan-diabetic rat (Jervis and Levin, 1966).

The importance of intraluminal nutrition in regulating mucosal growth and absorptive capacity in the small intestine of the rat has

been conclusively demonstrated by Gleeson et al., (1972a) after completely excluding segments of jejunum and ileum from intestinal continuity, by Thiry-Vella bypass operation; the effect of partial deprivation of luminal nutrition was also studied in jejunal segments that had been surgically transposed to a distal position in the intestinal tract. Villus height and total mucosal thickness were both reduced in the bypassed jejunal and ileal segments. Epithelial migration rate and turnover time were diminished in both bypassed jejunal and ileal segments. Jejunal transposition led to no morphological changes, but glucose absorption became significantly reduced.

In the present study intestinal transit of barium indicated that following right hemicolectomy, the duration of contact between chyme and mucosa of the upper intestine is reduced. Therefore, the chyme bathing the distal small bowel of operated animals is likely to contain more nutrients than normal, and to remain in contact with the mucosal surface longer than in control rats. Thus, mucosal hypertrophy may develop as a result of increased nutrition even though food intake is not increased, but other factors may also be involved.

Following subtotal colectomy, pair feeding studies demonstrated that hyperphagia was responsible for most of the enlargement in the lower third of the intestine, a finding which seems to support the suggestion that when intestinal transit is shortened the chyme reaching the distal small intestine contains more nutrients than normal.

In all animals subjected to subtotal colectomy the gut just above and below the ileorectal anastomosis (about 1cm either side) was thickened, and invariably loaded with faeces at killing. It would appear that the terminal part of ileum assumed a storage function, which may explain why the seromuscular coat of this part was increased after

subtotal colectomy.

Since food intake restriction, by pair feeding, did not decrease mucosal hypertrophy in the upper small intestine of rats with subtotal colectomy, it would seem that increased nutrition was not responsible for this enlargement. The factor most likely to be involved is increased pancreatic secretions. At present there is evidence that increased amounts of bile acids entering the duodenum stimulate secretion of pancreatic juice (Forrel, 1972). Following subtotal colectomy it is most likely that the watery diarrhoea which invariably follows the operation may be associated with excessive loss of bile acids, which could initially reduce the size of bile acid pool, leading to increased bile acid synthesis (Hofmann, 1967; Hofmann and Poley, 1972); so great as actually to over compensate, with the consequence that the quantity of bile acids entering the duodenum increases, thereby augmenting the secretion of pancreatic juice. This state of affairs is likely to persist as long as intestinal absorption remains poor, and the mucosa, particularly of the upper small intestine, may thus remain exposed to increased pancreatic secretions long enough to develop hypertrophy.

It is also likely that pair fed hungry animals will consume their ration very fast and remain without food for long periods. Thus, intermittent fasting, with spaced intake of large amounts of food, which is known to cause enlargement of the digestive tract in the rat (Fabry and Kujalova, 1960b; Febry, 1968) might partly account for enlargement of the upper small intestine in the pair fed subtotal colectomised rat.

(b) The 'Tissue Mass' or 'Self-Inhibition' hypothesis (Bullough, 1965), which postulated that the cells of an organ elaborate a circulating growth inhibitor, the concentration of which falls with partial ablation



due to loss of tissue mass, thereby permitting the remnant to grow until sufficient mass is regained to restore the concentration of inhibitor to normal levels. The existence of circulating growth inhibitor(s) in the intact rat, for example, is indicated by the Moolten and Bucher (1967) observation on hepatic regeneration: following partial hepatectomy, DNA synthesis, judged from  $^{14}\text{C}$ -thymidine incorporation, is greater in the operated than in the intact liver. However, DNA synthesis in hepatectomised rats paired to normals by a cross circulation is significantly less than in individually kept hepatectomised rats. Exchange-transfusion in the operated animal with blood from normal rats greatly inhibits DNA synthesis in the recipient animals, indicating that a substance/substances circulate(s) in the intact animal which inhibit(s) growth. On the other hand, the ability of blood from the hepatectomised rats to stimulate  $^{14}\text{C}$ -thymidine incorporation into hepatic DNA of the normal partners would seem to indicate the existence of a growth stimulator in the blood of partially hepatectomised rats.

The validity of the tissue mass hypothesis in connection with intestinal regeneration was tested (Tilson, 1972) by comparing the growth of villi after partial enterectomy against the growth induced by a major functional bypass of the gut without loss of tissue mass. The results conclusively demonstrated that villus hypertrophy can be induced without loss of tissue mass, suggesting that the mechanism initiating compensatory growth is not due to loss of an inhibitor elaborated by the resected segment. Thus, the growth of jejunal villi after bypass surpassed that observed after 75% distal bowel resection.

It must be stressed, however, that Tilson's results did not provide conclusive evidence against the tissue mass hypothesis. Since many factors seem to be capable of inducing the alimentary tract to grow bigger,

it would be necessary to exclude all other stimulators in order to evaluate with accuracy the effect of any given factor.

In the present study, increase in small bowel weight after colon bypass (without loss of tissue mass) was approximately the same as that after subtotal colectomy, indicating that loss of functional mass (or surface), rather than mere loss of tissue mass, was the main cause of this enlargement. On the other hand, after left hemicolectomy the absence of postoperative diarrhoea would seem to indicate that there was no appreciable loss of functional mass; may be absorption in the rat colon, like that in the human large intestine, takes place mainly in the proximal part. The occurrence of colonic mucosal enlargement after left hemicolectomy, therefore, can neither be explained on the basis of increased nutrition, nor on the functional demand hypothesis (*vide infra*), but seems to support the 'reduced self-inhibition' theory, although other factors could be involved.

(c) The Functional Demand Hypothesis propounded by Tilson and Wright (1970) proposes a feedback mechanism whereby the functional need for increased absorptive surface is communicated to the gut by means of a circulating stimulator of villus growth. This theory is based on the occurrence of villus hypertrophy in the shortened small bowel after bypass, where loss of absorptive surface, rather than reduction in tissue mass, is the major stimulus for compensatory growth. The existence of a circulating growth stimulator was indicated by the occurrence of villus hypertrophy in a defunctionalized (bypassed, self-emptying) blind ileum (Tilson and Wright, 1970), and seems to be confirmed by the demonstration of induced enterocyte hyperplasia in the unoperated twin in parabiotic pairs of rats in which one of the rats had partial small bowel resection (Tilson and Wright, 1971).

Since the alimentary tract can be stimulated to grow bigger by a variety of factors, more than one factor may be involved at any one time, depending on the prevailing conditions. In the present study the results seem to indicate that how much the remaining intestine enlarges after colon resection depends on the postoperative functional state: thus, after left colectomy intestinal function remained normal and bowel enlargement was confined to the mucosa of the remnant colon (excluding the caecum). Following right hemicolectomy, intestinal transit-time was reduced, diarrhoea developed, and the faecal dry matter output increased; all these indicate disturbance in bowel function. Compensatory growth involved both mucosa and seromuscular coat of the remnant colon, and mucosa of the small intestine, particularly of the lower third. With resection of both the proximal and distal colon, intestinal function was severely impaired, judging from the greatly reduced transit-time, watery diarrhoea and increased faecal dry matter output. The compensatory growth of the small bowel mucosa was more widespread and surpassed that observed after right hemicolectomy.

Thus it would appear that the extent of compensatory growth in the remnant intestine is determined by both the degree and nature of impairment of bowel function, which in turn determine the number of growth stimulating factors involved. For example the diarrhoea developing after subtotal colectomy, besides being more severe than that after right hemicolectomy, could have also been associated with: 1) marked water and electrolyte imbalance sufficient to cause increased secretion of antidiuretic hormone and mineralocorticosteroids; in which case the latter hormones could partly account for the mucosal hypertrophy (Kenney, 1962; Lebenthal et al., 1972; 2) excessive loss of bile acids sufficient to lead to increased bile acid synthesis, and augmented

secretion of pancreatic villus enlarging factors; 3) malabsorption of dietary energy, which may partly be responsible for induction of the hyperphagia that caused enlargement of the distal small intestine.

Whether or not mucosal enlargement was associated with increased absorptive cell mass cannot be deduced with accuracy from mucosal weight. This is because the mucosal mass obtained by scraping all mucosa down to the muscularis mucosae consists of three functionally different cellular compartments, viz.: 1) the villus columnar epithelium in the small intestine, or the columnar epithelium lining the flat mucosal surface of the colon; 2) crypt epithelial cells (and the intervillus epithelium in the small intestine); and 3) the loose cellular connective tissue, with many lymphoid cells, blood and lymphatic vessels, which forms the core of each villus and fills the interstices between the crypts, in other words the lamina propria. Changes in cell population in any of these compartments may affect the weight of mucosa. Since it has been indicated by histochemical studies that in the small intestine the crucial absorptive compartment is the villus columnar epithelium (Padykula, 1963; Dahlqvist, 1967), the mucosal mass obtained by scraping is not a good indicator of the actual absorptive cell mass.

However, microangiography and histological studies (Nylander and Olerud, 1962; Nylander, 1963) seem to indicate that in the absence of inflammation, mucosal hypertrophy is not associated with increased capillary proliferation in the lamina propria; the lymphoid cell population together with the lymphatics are also not likely to increase. In this case the change in mucosal mass would involve the absorptive villus epithelium (enterocytes) and the epithelial cells lining the intervillus ridges and crypts of Lieberkuhn. These two compartments are related: autoradiographic studies carried out after labelling nuclear

DNA of mitotic cells (e.g. with  $^3\text{H}$ -thymidine or  $\text{P}^{32}$ ) have demonstrated that cell division occurs in the crypts of Lieberkuhn (Leblond and Stevens, 1948; Cairnie et al., 1965); the newly formed cells migrate onto the villus surfaces (Leblond et al., 1948; Leblond and Messier, 1958; Loran and Crocker, 1963; Altmann and Enesco, 1967) to replace old enterocytes which are extruded at the tips of villi (Leblond and Stevens, 1948; Hooper, 1961). In the absence of inflammation, therefore, one may assume that changes in mucosal weight indicate changes in the functional cell mass.

However, this does not necessarily imply a functional relationship, i.e. the functional capacity of the intestine does not always change with the functional cell mass. The dividing cells in the crypts and those at the very base of villi, are believed to be functionally immature and incompetent (Leshner and Bauman, 1967); maturity develops as the cells migrate up the villi (Padykula, 1963, Dahlqvist, 1967).

Normally the epithelial cells proliferate very rapidly: the migration from the crypt mitotic zone up to the tips of the villi takes about 2 days in the rat (Leblond and Stevens, 1948; Bertalanffy, 1960) and in the cat (McMinn, 1954), about 3 days in the mouse (Leblond and Messier, 1958) and 3 - 6 days in man (Bertalanffy and Nagy, 1961; Lipkin, Sherlock and Bell, 1963; MacDonald et al., 1964). But in conditions associated with mucosal enlargement the rate of cell production and migration may be greater than normal. For example, in the small intestine of the lactating rat (Fell et al., 1963) and in the adapted remnant of the rat small intestine (Loran and Althausen, 1960) epithelial cell production and migration is more than doubled. In these and other conditions leading to decreased cell turnover time, one might speculate that the villus epithelium would be populated by functionally immature and incompetent cells - a condition likely to depress intestinal absorption. Wesser and Hernandez (1971), for example, observed

significant reduction in glucose transport in everted rat jejunal sacs despite an increase in mucosal mass; the activities of some mucosal enzymes (lactase, sucrase and maltase) were also significantly lower in homogenates of whole mucosa and isolated villus epithelium collected from jejunal remnants. The absorption of vitamin B<sub>12</sub> in the hypertrophic ileal mucosa of the guinea-pig (Mackinnon, 1973) was significantly increased after jejunectomy, but the uptake of cyanocobalamin (<sup>57</sup>Co) per unit cell mass (i.e. per mg of mucosal DNA) was decreased when compared to control segments; the marked increase in the number of enterocytes per unit length of villus margin in operated animals probably compensated for the functional incompetence, and accounted for the increase in B<sub>12</sub> absorption.

On the other hand, increased activity of unit mass of tissue may increase intestinal absorption despite reduction in mucosal mass. In semi-starved rats, for example, the small intestine markedly declines in both diameter and thickness (Kershaw, Neame and Wiseman, 1960), and develops mucosal atrophy (Wiseman and Neame, 1959); but in spite of these changes absorption of glucose and L-histidine in everted small bowel sacs, and in the *in vivo* perfused small intestine (Kershaw *et al.*, 1960), as well as the absorption of glucose and fat (Kujalova and Fabry, 1960) is increased. Dowling, Ricken, Laws and Booth (1967) also observed a marked increase in the absorption of glucose and water by everted jejunal sacs of rats previously maintained on kaolin diluted diet, but this increase in absorption was not associated with changes in intestinal weight, mucosal thickness or villus height. Similarly, the activities of some mucosal enzymes (leucine aminopeptidase, and several dehydrogenases) in the small intestine of bulk-fed rats have increased as judged by histochemical studies, without change in mucosal

mass (Ricken et al., 1965).

However, since mucosal hypertrophy seems to enhance absorption, e.g. of vitamin B<sub>12</sub> in the guinea-pig ileum after proximal small bowel resection (Mackinnon, 1972), of glucose in the rat's adapted ileal remnant (Bury, 1972), and of water and glucose in the ileum of rats previously subjected to either total colectomy (Wright et al., 1967) or partial small bowel resection (Dowling and Booth, 1967), it would appear that changes in mucosal weight (mass) indicate changes in the absorptive functional ability of the intestine. A number of experimental observations further support this concept. The activities of many mucosal enzymes in the rat jejunum and ileum: leucyl beta-naphthylamidase, arylsulphatase, beta-glucuronidase, beta-galactosidase, alkaline phosphatase, alpha-glucosidase, cytochrome oxidase and catalase have been found to decrease when mucosal mass (measured per cm of intestine) is reduced, following bypass operation; and they increase with mucosal hypertrophy (Gleeson, Dowling and Peters, 1972). Also, in vivo perfusion studies have demonstrated that whereas progressive deterioration in glucose absorption occurs in the bypassed atrophic jejunum (Gleeson, Cullen and Dowling, 1972), the hypertrophic jejunal or ileal segment showed increased glucose absorption (Dowling and Booth, 1967).

It must be remembered, however, that measurement of mucosal weight may give a false impression concerning the functional state of the intestine. Since variation in intestinal length affects mucosal weight, it is customary to measure mucosal weight per unit length of intestine, and to compare mucosal mass obtained from exactly the same level (or nearly so) in control and test animals, so as to avoid differences which may be caused by variations in villus size at different levels of

intestinal length (Warren, 1939; Wood 1944; Fisher and Parsons, 1950). However, it should be remembered that an apparent increase in mucosal weight, when measured per unit length of intestine, is likely to occur if there is shortening of bowel. It is known, for example, that the first response of bowel to distension, e.g. caused by intestinal obstruction, is contraction of the longitudinal muscles. This reduces the length of intestine, depending on the degree of obstruction: in the guinea-pig, for example, complete obstruction may cause the small intestine to contract to 25 to 20% of the entire length (Crane and Henderson, 1924; Trendelenburg, 1917). Acute experiments in the dog (Sperling and Wangenstein, 1935) have demonstrated that following complete obstruction of the terminal ileum the weight of the intestine immediately above the site of obstruction (measured per foot of intestine) was 114% greater than that of control segment; but when the shortening factor was eliminated the true gain was found to be only 34%.

In experiments involving surgical manoeuvre on the gut, partial obstruction may result from a bad intestinal anastomosis. This may give rise to mild chronic obstruction, not necessarily associated with intestinal dilatation. In such a situation anatomical changes in the wall of the bowel may develop as a result of supranormal intraluminal pressure caused by the obstruction. That structural changes in the wall of the bowel may develop even before there is bowel distension has been demonstrated: Sperling (1938) carried out experiments to ascertain what grade of intestinal pressure is necessary to produce anatomical changes in the gut wall; using closed ileal loops, it was demonstrated that for intraenteric pressures of 10 to 15cm of water (normal for dog ileum is 2 - 4cm of water) there was no distension of bowel, but at both these pressure levels there was congestion of bowel with petechial



haemorrhage on the antimesenteric border. In mild chronic obstruction not associated with intestinal distension, therefore, the mucosa may increase for a number of reasons: 1) because of oedema, and vascular congestion in the lamina propria; 2) because in the contracted state there would be more mucosal mass per unit length of intestine as a result of close packing of villi, even though total mucosal mass may not be increased; and 3) there may be actual mucosal hypertrophy or hyperplasia.

In the present study there was no evidence to suggest narrowing or inflammation in either the small or large intestine. Restoration of intestinal absorptive function, indicated by the disappearance of postoperative diarrhoea, was assumed to be a result of increased absorptive cell mass. This assumption was tested by comparing the size of villi and enterocyte density in operated and control animals (see following section).

---

(c) Villus Height and Enterocyte Density.

(c.i) After Right Hemicolectomy.

Villus height was studied in 8 hemicolectomised and 8 sham operated rats three months after surgery, when animals were 6 months old and weighed on average  $456 \pm 9$ g (operated) and  $470 \pm 12$ g (controls). Unoperated controls were not used because no significant difference in mucosal weight was demonstrated between these and sham operated animals, a finding which indicates that villus height and the number of enterocytes per unit length of villus edge are not likely to be significantly different in the two groups.

Whole sections were examined, under low power magnification, for

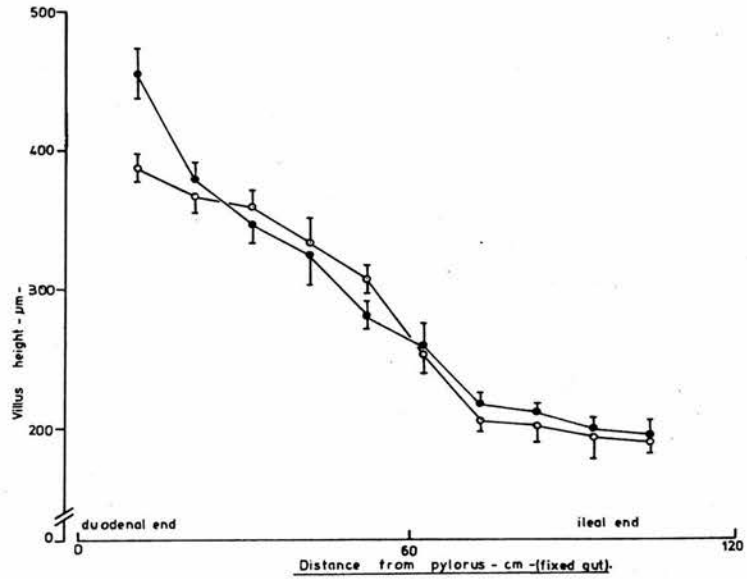


Fig. 25.

Villus height (mean  $\pm$  s.e.m.) after right hemicolectomy closed circles, and sham operation, open circles. Measurements were taken for 10 sites spaced at approximately equal intervals from the duodenal to the ileal end of the fixed small intestine in operated and control animals.

inflammation; none was found in all the sections taken for villus height and enterocyte density measurements.

The length of villi in any three consecutive segments did not differ significantly. For this reason results were pooled for every three successive segments and mean villus heights obtained, in each animal, for 10 levels. These represented 10 sites along the length of the small intestine spaced at regular equal intervals from the pylorus to the ileal end. Since the length of the fixed small intestine in hemicolectomised and in control rats was approximately the same (the average being  $109.3\text{cm} \pm 1.2$  in operated, and  $108.9\text{cm} \pm 1.1$  in control, rats), the 10 sites were, therefore, spaced at nearly the same intervals from the pylorus, in operated and in control animals.

When villi from three consecutive segments in operated rats were compared to the villi from similar control segments, a 2-way analysis of variance revealed no significant difference in villus height for all 10 sites. Similarly, when the mean villus heights for every three consecutive segments in each hemicolectomised rat were compared with control values, a paired t-test showed no significant difference for all the 10 levels (Fig. 25); however, the villi in the first proximal segment were probably taller in operated than in control animals,  $p < 0.05$ .

#### Enterocyte Density.

To find out how consistent epithelial cell counts were, 10 slides were counted twice. The duplicate counts differed from each other by 2.01% (range 0 - 5.0%), which was considered satisfactory.

Results were pooled for every three successive segments as described for villus height. When enterocyte counts from the three segments in operated rats were compared to similar control segments, a 2-way analysis

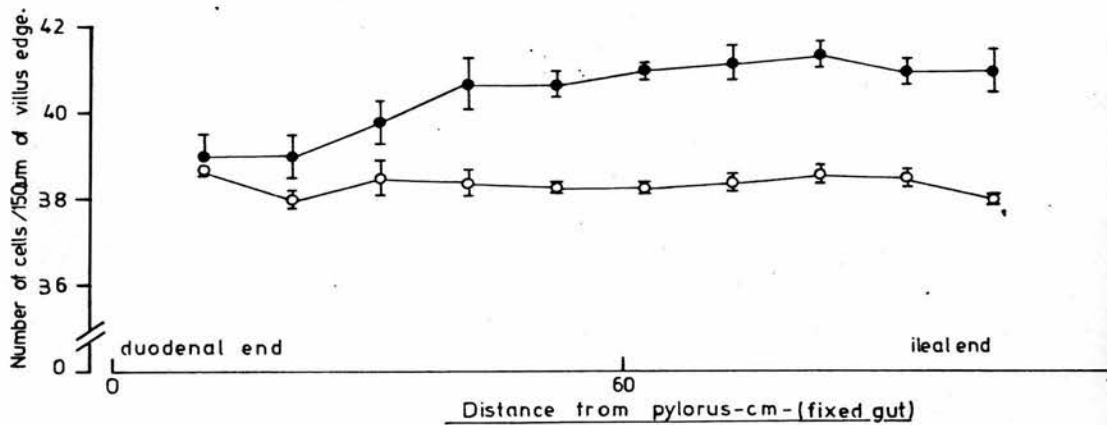


Fig. 26.

Enterocyte density (mean  $\pm$  s.e.m.) after right hemicolectomy (above) or sham operation (below). Measurements were taken for 10 sites spaced at approximately equal intervals from the pyloric end of the fixed small bowel in operated and control animals.

of variance showed significant differences in all but the first two proximal levels representing a segment of upper intestine approximately 21cm long (measured from the duodenal end of the fixed gut). Similar significant differences were observed when the mean enterocyte density measured for every three successive segments in each operated, and control animal were compared in a paired t-test (Fig. 26); the difference between operated and control values after the third level from the duodenal end is highly significant,  $p < 0.01$ ; although the increase in enterocyte counts was small, averaging 6 to 7%. At the third proximal level (Fig. 26) t-test reached  $p < 0.05$  significance.

(c.ii) Following Subtotal Colectomy.

Villus height was studied in 8 operated and 8 sham operated rats, three months after surgery, when the animals were approximately 6 months old and weighed, on average,  $429^{+11}g$  (operated) and  $463^{+8}g$  (controls). The length of the fixed small intestine was about the same in the two groups, the mean value being  $108.4cm^{+0.9}$  in operated, and  $109.1cm^{+0.3}$  in control, animals.

As in the right hemicolectomy group, the villi in any three consecutive segments were of approximately the same height. Results were pooled and analysed as described for hemicolectomy. The villus height at the first 4 proximal, and the last distal, levels (Fig. 27) i.e. about 44cm of upper intestine and 11cm of terminal ileum, were 10 to 12% taller in colectomised than in control rats. At each of these levels t-test reached  $p < 0.01$  significance. Similar significant differences were revealed by a 2-way analysis of variance. This was still the case when villus heights at all the 10 levels were compared between operated and control animals.

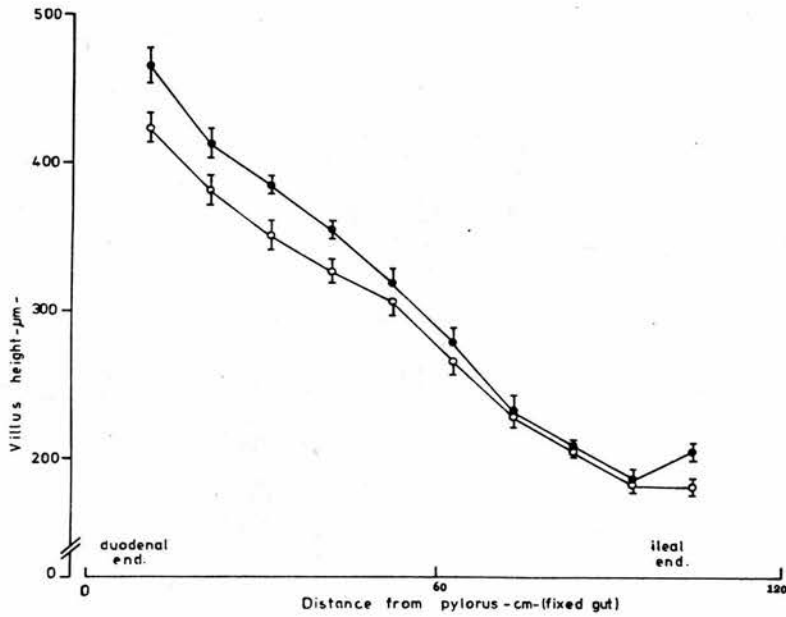


Fig. 27.

Villus height after subtotal colectomy (above) and ileal transection and anastomosis (below). Measurements were taken at 10 sites spaced at approximately equal intervals from the duodenal to the ileal end of the fixed small gut in operated and control animals. Values represent the mean and s.e.m.

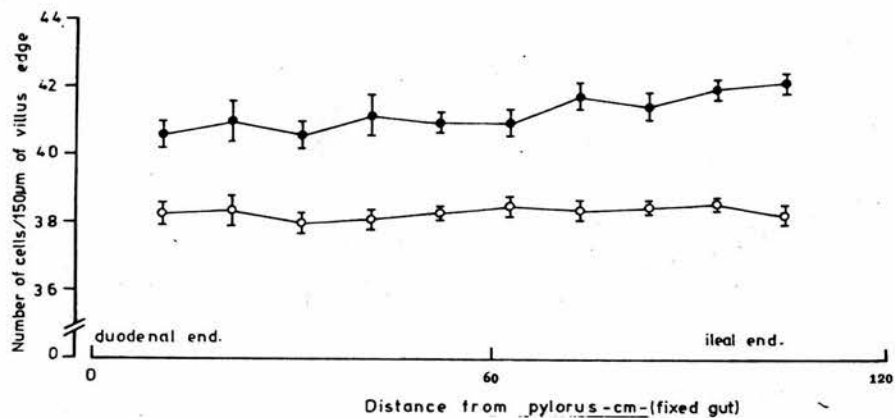


Fig. 28.

Enterocyte density (mean  $\pm$  s.e.m.) after subtotal colectomy (above) and ileal section and anastomosis (below) measured at 10 sites spaced at approximately equal intervals from the pyloric to the ileal end of the fixed small gut in operated and control animals.

### Enterocyte density.

Likewise, it was found that the number of enterocytes per 150 $\mu$ m of midvillus margin, all along the small intestine, was increased after subtotal colectomy (Fig. 28). Although the increase was small (averaging 7% at each level) t-tests reached  $p < 0.01$  significance at each point.

---

### Discussion.

An ideal way to ascertain whether a change in mucosal weight is associated with alteration in the functional cell mass is to determine the enterocyte population. At present, however, there is no method by which this could be done accurately. Calculation of cell population based on nuclear counts taken from microtome sections (Ernest et al., 1942; Marrable, 1962) are unsatisfactory because of the over-estimation of cell number which occurs when counts are made (Abercrombie, 1946; Clarke, 1968). Besides, such a measurement when applied to the intestinal villi is of one dimension of a three dimensional structure. In species which have leaf-shaped villi, e.g. the rat (Wood, 1944; Barker et al., 1963), calculations based on nuclear counts would give a poor estimate of the enterocyte population. This is because there may be an increase, or reduction, in enterocytes wholly undetectable by counting the number of cells in the villus column from base to tip. For example, Clarke (1974) observed that the villus column size in the rat terminal ileum after 5 days starvation was unaltered, but the circumference of the villi at their bases (calculated from nuclear counts in horizontal sections through the mucosa) was reduced by one third.

On the other hand since increase in enterocyte population is likely to affect the entire villus surface equally on its three dimensions,



counting of enterocytes along the villus margin may provide an idea about the magnitude of change in the functional cell mass. For the purpose of determining whether increase in mucosal weight is associated with increased functional cell mass, such measurement would at least give an indication. However, if the functional cell mass (weight) increased because of enterocyte hypertrophy rather than hyperplasia, cell counting would not detect the change. In such a situation it would seem likely that villus enlargement would occur as a result of enterocyte hypertrophy (Dowling and Booth, 1967b). By measuring villus height and counting the enterocytes along the villus margin, one is certain to detect changes in the functional cell mass. When these two measurements are combined with measurement of mucosal weight, the obtained results, when put together, enable one to predict with reasonable accuracy the functional ability of the intestine.

In this work enterocyte counting was confined to 150 $\mu$ m of the midvillus edge for a number of reasons: 1) Enterocytes are evenly packed along the midvillus margin in all villi taken from any level along the intestine; 2) In contrast, towards the villus tip it is usual to find gaps in the enterocyte lining, and the cells are generally not as regularly packed as they appear along the midvillus edge. 3) Towards the villus base, epithelial cells become more closely packed together; in thick sections they may actually appear one on top of the other, a situation which makes counting difficult and inaccurate. 4) Unless the same length, and approximately the same level, of villus edge is covered each time cells are counted results are likely to be unreliable. This is because the number of enterocytes per unit length of villus margin decreases as one moves towards the villus tip. In the present study, for example, the change in enterocyte density with distance from

the villus base was investigated by examining 100 villi taken from 10 different sites along the intestine of a normal rat. The average number of enterocytes per 150 $\mu$ m of top, mid and basal villus edge were 34.8, 38.9 and 44.1, respectively. The mean enterocyte density remained the same (within 2 cells difference) for the top, mid and basal villus margin all along the intestine, as judged from the 10 sites studied.

One way to avoid errors caused by change in enterocyte density along the villus edge is to count all epithelial cells from the base to the tip of the villus. By so doing, however, one obtains results which are probably not likely to give an accurate indication about changes in the absorptive cell mass; this is because the enterocytes at the very bottom of the villi are likely to be functionally immature (Leshner and Bauman, 1967; Padykula et al., 1962). On the other hand, by confining epithelial cell counting to the midvillus edge one is certain of including only functionally active cells.

In the present study the mean villus height after right hemicolectomy did not increase despite a 6-7% increase in enterocyte density. A similar situation has been reported by Porus (1965) who observed 50% increase in the number of enterocytes per unit length of villus margin, in human jejunal remnants; but despite this marked hyperplasia there was no enlargement of villi. Knudtson et al., (1962) have also reported a marked increase in crypt cell production (in the dog's small intestine after massive small bowel resection) which was not associated with increase in villus height. This is likely to be the case if the enterocytes are reduced in size or the life span of adult villus epithelial cells is shortened (Symons, 1965). In the small intestine of lactating rats, for example, increased crypt cell production may be associated with a slight reduction in the life span of enterocytes (Fell et al., 1963);

but the villi enlarge because cells are produced at a rate higher than they are lost. After right hemicolectomy it is possible that increased cell production maybe does not exceed cell loss; shortening of the life span of enterocytes could be effected through inhibition of protein synthesis by villus producing factors found in stagnant bile (Altmann, 1974). Stagnation of bowel contents in the lower small intestine, in this study, has been indicated by barium sulphate transit studies.

Although the increase in enterocyte density after subtotal colectomy was about the same as that after right hemicolectomy, the villus height in the upper 44cm of intestine and 11cm of terminal ileum was significantly greater than in the control segments. Enlargement probably occurred as a result of both enterocyte hyperplasia and hypertrophy.

The location along the small intestine of marked increase in enterocyte density after right hemicolectomy, and of villus height and enterocyte density after subtotal colectomy, corresponded with that of increase in mucosal weight, a finding which would seem to confirm the idea that in the absence of inflammation and all other factors likely to cause a false increase in mucosal mass, changes in mucosal weight indicate changes in the functional cell mass. The present results show that the rat's small intestine adapts to the absence of the caecum, or the entire large bowel, by increasing its absorptive cell mass. The restoration of intestinal absorptive function, as indicated by the disappearance of postoperative diarrhoea, in this study is attributed to the increase in absorptive cell mass.

---

PART 5.BARIUM SULPHATE ( $\text{BaSO}_4$ ) RECOVERY STUDY.Introduction.

One aim of the work presented in this thesis was to study, in the rat, intestinal function following removal of part or all of the colon, by measuring absorption of nutrients from the intestinal tract of unanaesthetised animals. Three general methods can be employed:

(a) Whole Animal Balance Studies Without Inert Marker (Conventional Balance Method).

The measurement of intestinal absorption by the conventional balance method is done by determining the intake and excretion, in faeces, of the substance, or nutrient under investigation; the difference between intake and excretion gives the amount absorbed. However, balance studies without a marker involve procedures which are not only laborious but also inaccurate. The following example illustrates the difficulties encountered.

Consider an experimental animal to which is administered orally a quantity 'Q' grams of a certain dietary substance. The fate of this substance may be specified: if 'E' grams are recoverable from the faeces and 'r' grams remain in the lumen of the intestinal tract, then the amount absorbed 'A' =  $Q - E - r$ . The first problem encountered in conventional balance studies, therefore, is to find ways of eliminating the unknown quantity 'r' of the material present and unabsorbed in the intestinal lumen. One way by which this problem can be solved is to feed the experimental material for only a specific short period, and to identify the faeces corresponding to that period. This has been

achieved by feeding a visible faecal marker, such as carmine (Bergeim, 1926; Reifenstein, Albright and Wells, 1945; Stanley and Cheng, 1956) or brilliant blue (Sharpe and Robinson, 1970), at the beginning and end of the experimental period, and then collecting all faeces lying between the two markers.

However, in order to minimize the inaccuracies and difficulties in isolating small "segments" of stools derived from a few meals, and to compensate for the variations in the daily stool quantity, particularly if the subject has irregular bowel habits, prolonged feeding and stool collection periods are often necessary (Bergeim, 1926; Stanley and Cheng, 1956). Thus, the cost and labour involved are usually great. In spite of this, collection of faeces is never complete. In human studies, for example, collection of faeces is usually incomplete because of loss on toilet paper and in general manipulation (Whitby and Lang, 1960; Rose, 1964).

Additional problems arise when dealing with small laboratory animals. If metabolism cages with the diet compartment removed from the main cage are not used, then one must contend with spillage of food into the faeces. Separation of food from faeces is inexact unless hard pellets are voided. Furthermore, unless the animals are restricted, most will eat faeces; the rabbit and the rat, for instance, are coprophagic animals (Kulwich et al., 1953; Eden, 1953; Herning and Hird, 1972). All methods of restriction are open to some criticism concerning the physiological and psychological state of the animals. Restricting the movement of rats or mice, for example, inhibits growth (Kotb and Luckey, 1972). From all these it is clear that results obtained by the conventional balance method are not likely to be accurate.

(b) Whole Animal Balance Studies With Inert Marker (Inert-Indicator Method):

In general terms, the inert-indicator method is a balance study in which the use of an inert marker in the diet makes it possible to replace total quantitative collection of faeces by random sampling. The criteria generally accepted for an ideal marker (or indicator) are: non-absorbability, no toxicity, the inability to induce physiological or psychological effects, uniform dispersibility relative to the faecal constituent of interest in a particular investigation, rapidity in attainment of a steady state (in which, on the average, excretion of the marker equals intake) and ease of quantitative measurement (Davignon, Simmonds and Ahrew, 1968; Kotb and Luckey, 1972).

If a marker with the above qualities is ingested daily, in constant amounts, the concentration of the substance in the faeces should increase rapidly to reach a plateau. Similarly, the daily output of the marker should rise to a plateau level. When a steady state for balance is attained, in which the amount of marker retained in the intestinal tract (the pool size) has become constant, the excretion in the faeces should quite closely approximate the intake. Such an ideal state was attained in 28 patients, in whom the concentration of chromic oxide ( $\text{Cr}_2\text{O}_3$ ) in the faeces reached a plateau in four days, and the mean daily output during the steady state was 90% (or more) of the mean daily intake (Davignon *et al.*, 1968).

When a steady state has been reached, the daily faecal excretion 'E' of any component of the stools can be calculated from the daily intake 'I' of the indicator, and the concentration 'Cs' of the component and the concentration 'Is' of the indicator in the same stool by the formula:

$$E = \frac{I \cdot Cs}{Is} \quad (\text{Stanley and Cheng, 1957}).$$

The amount of the component absorbed, as in the conventional balance studies, is given by the difference between intake and excretion. The following example illustrated the point:

Suppose it is desired to determine the absorption of dietary fat in a rat whose daily food intake and faecal output are indicated in the table below.

Fat and Indicator content in the rat diet and faeces; and the daily food intake and faecal output in a normal rat.

	<u>Food Dry Matter.</u>	<u>Faecal Dry Matter.</u>
Fat content, g/100g dry matter.	4.00	1.50* (= 'Cs')
Indicator (barium) content g/100g dry matter.	0.20	0.80 (= 'Is')
Daily food intake (g) dry matter	20.00	Daily faecal output. 5.00g dry matter

\* All except this are true values.

The daily barium intake 'I' =  $\frac{20.00 \times 0.20g}{100} = 40mg$ ; and the daily output =  $\frac{5.00 \times 0.80g}{100} = 40mg$ , indicating that the marker is not absorbed at all.

The amount of absorbed fat may be calculated, using the conventional balance method, as follows: Daily fat intake =  $\frac{20g \times 4g}{100} = 800mg$ ; and the daily faecal fat output =  $\frac{5 \times 1.5}{100} = 75 mg$ . The amount of absorbed fat would be  $800 - 75 = 725mg$ .

The same information regarding fat absorption would be obtained by using the formula  $E = \frac{I \cdot Cs}{Is}$ . Thus, faecal fat output

$$'E' = \frac{0.04 \times 1.50g}{0.80} = 75mg;$$

and the amount of absorbed fat being  $800 - 75 = 725mg$ .

The above derivations assume the dry basis analysis of food and faeces, and the excretion, or absorption, is therefore for the dry matter

of food component.

When using an ideal marker, one can deduce the intake of food corresponding to a certain collection of faeces, even if the actual food intake is not measured, and the scatter is a nuisance. To do this, the marker would be added to the diet in a ratio calculated for the quantity, either of the total food or of any given nutrient. The total faecal output would be measured by quantitative collection, or indirectly by the use of a collection marker. Analysis for the quantity of marker present in the faeces will allow calculation of the quantity ingested. Results obtained by this method give a more accurate estimate of the ingested amount of food or marker than estimates obtained by actually measuring the intake (Kotb and Luckey, 1972).

The measurement of intestinal absorption, and of excretion in faeces, by the inert-indicator method, therefore, provides the greatest possible precision in whole animal studies; although trials in dairy cattle (Kane, Jacobson and Moore, 1950), in the rat (Schürch, Lloyd and Crampton, 1950) and in man (Irwin and Crampton, 1951) showed no significant difference between digestibility coefficients obtained by the use of chromic oxide ratios and those calculated by the conventional balance method. The main advantages sought when the inert-indicator method, rather than the conventional balance study, is applied include: the ability of the investigator to avoid inaccuracies resulting from incomplete faecal collections and/or inaccurate estimate of food intake; and the possibility of replacing total quantitative collection of faeces by random sampling, thereby cutting down labour and cost.

It must be noted, however, that in animal studies, it is important that the random sampling of faeces employed for the inert-indicator method, should be done in such a way that the samples taken for analysis



are representative of the 24-hour faeces. Diurnal variation in the concentration of inert markers in the faeces, e.g. the concentration of chromic oxide, or lignin, in the faeces of the dairy cow (Kane et al., 1952) and in the heifer (Elma et al., 1959) has been observed. Thus, the content of chromic oxide in the faeces, for example, rose to the highest point at 0900 hours, and the lignin peak occurred at about 2000 hours (Kane et al., 1952). Diurnal variation of chromic oxide concentration has also been observed in the goat faeces (Kameoka, Takashishi and Morimoto, 1956) in pig stools (Clawson, Reid, Sheffy and Willman, 1955; Moore, 1957) and in chicken droppings (Meuller, 1956). Thorough mixing of chromic oxide with the ingredients of a pelleted diet did not eliminate or significantly reduce the diurnal variation in cattle even when freely fed (Elma et al., 1959).

Furthermore, when studying excretion of dietary components in the faeces of coprophagic animals, improper sampling of faeces may cause inaccuracies as a result of diurnal variation in the composition of stools. The rabbit, for example, excretes two types of faeces - a hard type of pellet and soft stools. The latter are for eating and are very rich in protein content, vitamin B complex (Eden, 1953) and in volatile fatty acids (Hernning and Hird, 1972); and are voided during the night, in domesticated rabbits (Kulwich et al., 1953; Eden, 1953; Hernning and Hird, 1972), the opposite rhythm applying in the wild animal (Hernning and Hird, 1972). In any animal study where the inert-indicator method is applied, it is therefore important that the faecal samples taken should be representative of the 24-hour stools.

### (c) Techniques Involving Intubation of the Intestine.

These methods are based on the examination of samples of intestinal

contents removed by aspiration from the intubated lumen (Parsons, 1968). When it is desired to measure absorption from a segment of intestine, e.g., to determine the site or segment from which a given substance is absorbed, intubation techniques become useful in man. However, in the unanaesthetised rat, intestinal absorption studies by this method are not feasible. Even in the anaesthetised animal, such studies can hardly be used to deduce the actual amounts absorbed by the whole animal. This is because in the anaesthetised animal, or isolated pouches, absorption rates rarely approach those of the whole normal animal. Such studies are valuable for discovering the detailed mechanism of absorption, rather than for measuring impairment or improvement affecting whole animals. Since the aim in this work was to study absorption in whole animals, further discussion on the techniques involved in intestinal intubation is, therefore irrelevant.

#### Choice of Method.

The inert-indicator method was chosen so as to achieve great precision, and incidentally avoid the labour and wastage of time involved in conventional balance studies.

#### Choice of Marker.

The markers used in the determination of faecal excretions have been substances having the properties outlined on page 85., and have been chiefly the metal oxides, Chromium Sesquioxide,  $\text{Cr}_2\text{O}_3$ , (Kane *et al.*, 1950; Schurch *et al.*, 1950; Irwin & Crampton, 1951; Stanley and Cheng, 1957; Rose, 1964; Sharpe and Robinson, 1970), Titanium Oxide,  $\text{TiO}_2$ , (Fournier, 1950; Fournier and Dupuis, 1953), Ferric Oxide,  $\text{Fe}_2\text{O}_3$ , (Bergein, 1924; Bergein, 1926; Gallup, 1928) and the mineral salt - Barium Sulphate,  $\text{BaSO}_4$ .

Barium Sulphate ( $\text{BaSO}_4$ , M.W. 233.43) is a fine, heavy, white, odourless, tasteless powder, free from grittiness; almost insoluble in water and organic solvents; very slightly soluble in hydrochloric and nitric acids and in solutions of alkalis and many salts (Martindale, 1972). Barium sulphate has long been used as a radiopaque medium for digestive tract studies in man (Merk Index, 1968); and as an indicator in nutrient utilisation studies after it was established that it is not toxic, not absorbed from the digestive tract and is completely recovered in the faeces of man (Figueroa *et al.*, 1968). In chickens, 0.5% of barium sulphate in the diet has no toxic effect (Whitson *et al.*, 1943).  $^{131}\text{BaSO}_4$  was not absorbed from the intestinal tract of mice and dogs (Seife, 1962). Studies in the rat showed that barium sulphate is not toxic when administered intragastrically in doses ranging from 1 to 160g/Kg body weight; large doses, 307 to 364g/Kg body weight were needed to cause death (Boyd and Abel, 1966); even then death was due to stomach rupture, or to bowel obstruction.

In the present study barium sulphate was chosen because it was found to mix readily with ground rat food. Chromic oxide tended to stick to the sides of the container, leading to losses, and was not chosen for this reason.

---

#### Measurement of Barium Sulphate in Food and in Faeces.

The measurement of  $\text{BaSO}_4$  by the gravimetric method (Dick, 1967; Figueroa, Jordan and Basset, 1968), or by emission flame photometry (Dick, 1969), is tedious and time consuming. In this work a rapid method of measuring barium sulphate by X-ray fluorescence spectrometry (XRF) was adopted.

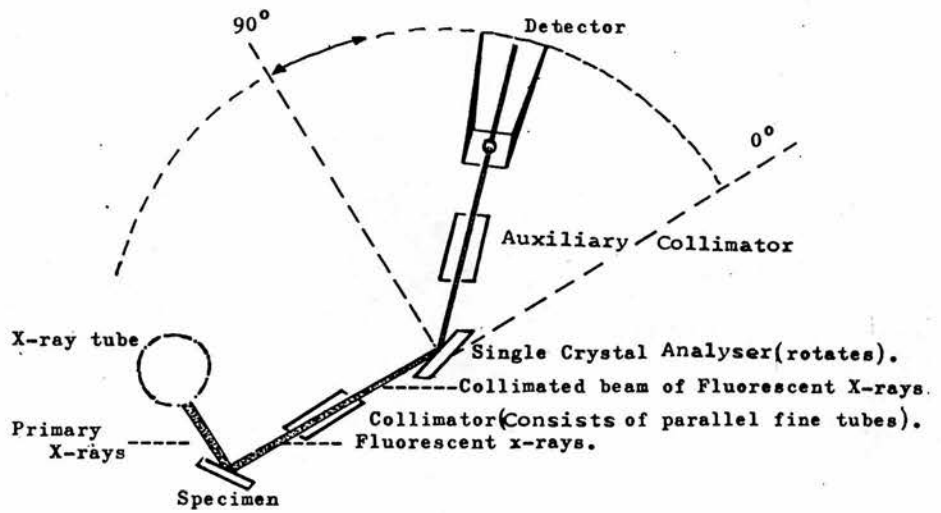


Fig. 29.

Basic geometry of X-ray emission spectrometer.

## X-ray Fluorescence Spectrometry (XRF).

This method is based on x-ray emission analysis, which has become one of the most potent tools available to the analyst for the study of metals and other massive samples, and is accepted as being highly accurate for elemental analysis (Jenkins and De Vries, 1967). However, there are limitations to this method: the analysis of light elements, i.e. those with atomic numbers less than 11 (Sodium) so far is impracticable, and those below 20 (Calcium), only with some difficulties (Ewing, 1969).

### Principle of Method.

When a beam of x-rays is incident upon a target sample, depending on the energy of the incident radiation, i.e. the wavelength, and on the nature of the sample, the x-rays may cause emission of energy from the sample matrix in the form of fluorescent x-rays. A sample is usually excited by irradiation with a beam of primary x-rays of greater energy than the secondary x-radiation which it is desired to excite in the sample. Because of the need for high energy primary radiation, a tungsten-target tube is generally employed. Each heavy element in the sample is excited to emit x-ray frequencies (Wavelength) characteristic of the element. A collimated beam (all rays parallel) is then obtained by passing the fluorescent x-rays through a bundle of metal tubes (collimator), or through a series of narrow slits, onto an analysing crystal. The analysing crystal is a filter consisting of an element, or its compound, which absorbs x-rays, and can be rotated in such a way that only the desired wavelength is allowed to pass onto an auxiliary (secondary) collimator as a diffracted beam of x-rays (Fig. 29). The emerging x-rays may be detected photographically, or electrically by

means of the ionisation they provide in gas; by scintillation counting or by the photoelectric effect produced in the semiconductor elements germanium or silicon.

By setting the analysing crystal and the secondary collimator at chosen angles, the characteristic wavelength of radiation emitted by individual elements in a given sample can be separated and measured.

In the present study, it was hoped that the absorption of calcium, chloride, potassium, magnesium and sodium could be studied by estimations on the same samples as used for barium estimation. Thus, the absorption of dietary components from the alimentary tract, and the losses of electrolytes in the faeces after partial or total colectomy could be studied simultaneously with much ease.

#### Design of Experiment.

Because of lack of information regarding the suitability of barium sulphate as an inert-indicator for digestive studies in the rat, it was necessary to find out what happens when this marker is fed to rats over a number of days. A scheme was designed to provide answers to the following questions:-

When rats start taking barium sulphate containing food and keep taking it,

- (a) How long does it take to reach a steady state of excretion in the faeces of barium sulphate, and how steady is it when reached?
- (b) What concentration does barium sulphate reach in faeces?
- (c) What recoveries of total barium sulphate taken can be expected?
- (d) How long does it take for excretion to stop when unmarked food is started again.

Answers to these questions would not only provide information concerning

the suitability of barium sulphate as an inert marker to be used in the rat, but would also indicate when to take faecal samples for analysis.

#### Animals Used.

24 male albino rats of the Wistar strain were used. They were obtained, at two months of age, from the same source, and were kept in pairs under the same conditions, as described for animals used in colectomy studies.

#### Preparation of Barium Sulphate-marked Food.

Supplies of diet GR3.EK ( page 9. ) were taken to the Poultry Research Centre, Kings Buildings, Edinburgh, for grinding and pelleting. To a portion of finely ground diet, barium sulphate ( $\text{BaSO}_4$  : 58.84% Ba, 13.74% S and 27.42% O) was added to give a mixture containing 0.20% Barium w/w. The blended diet was mixed in a Whobert food mixer ( see page 96 for uniformity of mixing). Water was added, 400ml/2.5Kg of the ground diet, and the food was worked up in the mixer to a semipaste state. Food pellets were prepared using a pelleting attachment fitted to the mixer, and were dried overnight in a hot dry air oven at 125°F. Unmarked pellets were prepared in a similar manner. Pelleting was necessary because food supplied to animals in the pellet form tended to cut down wastage through scattering.

#### Procedure for Recovery of Barium Sulphate.

The food intake and the 24-hour pooled faeces of each pair of rats were measured daily for three weeks. Since the food in pellets containing barium was more finely ground and contained less water, 4.34% water compared to 8.42% in the commercially prepared pellets, it was thought necessary to condition the animals to the altered diet in

order to ensure a steady intake. For this reason, animals were fed, during the first week, unmarked food pellets which were similar to those containing the marker. Throughout this week all animals maintained an intake that was within normal range, 39.0 to 43.4 grams of dry food per pair per day. Animals were fed barium containing food during the subsequent week. Unmarked pellets were reintroduced at the beginning of the third week, and were eaten daily for 7 days. It was assumed that the concentration and excretion of barium in faeces would reach a steady state within the seven days of feeding the marked diet.

The daily intake of food dry matter was measured for each pair of rats by the same method as described on page 12. The 24-hour faeces were separated from spilled food and collected for each pair, and were dried to constant weight in a hot air oven at 85°C. constant temperature.

#### PREPARATION OF SAMPLES FOR ANALYSIS.

##### Stools.

When dried, all stools collected during the first week were ground in a coffee grinder and mixed together in a bucket. From this pooled stool, a sample was taken for determination of the baseline amount of barium in normal unmarked stools. Three additional samples were taken, to which barium sulphate was added to give blended faecal samples containing 0.58%, 0.88% and 1.76% barium w/w. Since the accuracy in detecting a substance from a sample matrix depends not only on the sensitivity of the method used, but also on whether there are other substances in the sample which interfere with the detection, recovery of added barium was studied in order to assess the accuracy of using XRF for determining barium content in rat faeces.

Faeces collected during the week of feeding barium containing food,



and during the subsequent week of refeeding unmarked pellets, were kept in daily lots for each pair of rats. These were ball milled and analysed for barium, calcium, chloride, potassium, sodium and magnesium content.

#### Diet samples.

After the diet was thoroughly mixed with barium sulphate, samples were taken: one from food lying at the bottom of the food mixer, another from the top, and a third from midway between. These were dried to constant weight and prepared for analysis of barium content. Results were used to check the uniformity with which the Whobert mixer mixed barium with the diet. The content of barium in the market diet, dried to constant weight, was also determined from these samples. A blank food sample was analysed to determine the baseline amount of barium in unmarked diet. Additional samples were taken to which barium sulphate was added to give mixtures containing 0.58%, 0.88% and 1.76% barium w/w. Recovery of added barium was done.

Analysis of samples was carried out at Roslin Laboratory, Midlothian, using an X-ray fluorescence spectrometer with a single flat crystal system and a gas flow proportional counter.

### RESULTS.

#### Recoveries on added barium sulphate.

The baseline amount of barium in unmarked faeces, determined from a single sample, was 55 parts per million (ppm). Values obtained for marked stools were, therefore, corrected by subtracting the baseline amount from the observed amount. Thus, recoveries on added barium were

Table 14.

Concentration and excretion of barium in faeces during, and soon after, the week of feeding barium sulphate.

Day.	Daily barium intake (mg).	Concentration of barium in faeces mg/g dry faeces.	Daily barium excretion in faeces (mg)	Daily output as % of intake.
	mean (range)	mean (range)	mean (range)	mean
1	88.4 (64.2-147.4)	2.7 (1.8-4.7)	29.2 (18.3-47.1)	32.9 %
2	85.2 (68.0-109.1)	7.4 (7.3-7.7)	72.1 (56.5-87.3)	86.6 %
3	83.2 (69.2-91.8)	7.7 (7.5-8.0)	80.0 (66.8-109.1)	96.1 %
4	84.2 (66.2-98.0)	7.7 (7.6-7.9)	87.9 (67.2-100.4)	104.4 %
5	85.4 (73.8-103.8)	7.6 (7.5-7.8)	77.1 (62.9-106.0)	90.2 %
6	91.4 (73.2-122.8)	7.6 (7.5-7.9)	86.0 (78.9 -95.2)	94.2 %
7	95.3 (73.0-129.6)	7.6 (7.4-7.8)	88.6 (74.2-104.0)	91.0 %
8	- Nil -	5.6 (4.9-6.4)	53.3 (43.4- 75.5)	-
9	- Nil -	0.5 (0.4-0.8)	6.3 ( 4.4 - 8.4)	-
10	- Nil -	0.05 (0.0-0.1)	2.2 ( 0.4 - 3.8)	-
11	- Nil -	Baseline, $60 \pm 5$ ppm.	-----	-

99.6%, 100.8% and 100.7% for stool aliquots containing 0.58%, 0.88% and 1.76% barium w/w, respectively.

The baseline content of barium in unmarked food was 20 ppm; the corrected recoveries on added barium being 99.0%, 101.2% and 99.5% from the food samples containing 0.58%, 0.88% and 1.76% Ba. w/w, respectively. The three samples which were taken to check uniformity in the mixing between barium and food, when dried to constant weight, contained 2.16mg, 2.19mg and 2.21mg per gram. These values represent 108.0%, 109.5% and 110.5%, respectively, of the amount of barium that was added to the diet at mixing (2.0mg Ba/gram of diet). It should be remembered that when dried to constant weight, the blended diet lost, on average 8.3% of its weight at mixing. Therefore, the slight increase (8 to 10%) in the content of barium observed in dried blended food samples can be explained by the loss of weight due to water evaporation. The mean of the three values i.e. 2.18mg/gram of dry blended diet, was used in calculating daily barium intake.

Although the number of observations on recovery of added barium is too small to allow meaningful statistical conclusions to be drawn, there is, at least, an indication that the measurement of barium in rat faeces and in the rat diet can rapidly be done by the XRF method with good results. Furthermore, it would appear that the Whobert mixer, in this case, provided even mixing between barium and the diet. However, more observations are needed before definitive statistical conclusions can be made.

#### Steady state and recoveries on fed barium.

The concentration of barium in faeces rose rapidly, reaching a plateau level on the third day of feeding barium sulphate (Table 14)

Table 15.

Total Barium Intake and Excretion.

Pairs of rats:	Total barium intake: (intake: mg)	Total barium excreted: (output: mg)	output as % of input.
A	529.4	562.6	106.2
B	577.8	541.2	93.6
C	529.4	539.0	101.8
D	646.6	593.9	91.8
E	595.4	587.6	98.6
F	615.8	571.9	92.8
G	679.6	627.6	92.3
H	651.0	581.6	89.3
I	826.6	669.6	81.0
J	601.4	589.1	97.9
K	524.2	548.6	104.6
L	546.8	557.2	101.9
Mean			96.02 %
s.e.m.			2.08

Measurements in stools include barium excreted since the first day of feeding the marker to the day barium reached baseline amounts. Low recoveries in pairs H & I cannot be explained.

Table 16.

Daily Output of Electrolytes in Normal Rat Faeces

Day	Calcium m-moles/day	Chloride μ-moles/day	Magnesium μ-moles/day	Potassium μ-moles/day	Sodium μ-moles/day
	mean ± sem.	mean ± sem.	mean ± sem.	mean ± sem.	mean ± sem.
1	10.4±0.03	268.0±30.7	832.0±23.0	337.9±24.3	418.1±24.0
2	10.3±0.03	128.6±16.2	710.1±48.1	504.1±29.9	301.9±29.2
3	8.7±0.02	134.9±15.4	730.9±42.1	383.4±12.1	278.0±22.4
4	10.0±0.02	143.4±10.1	844.0±30.8	377.6±11.4	399.9±29.4
5	9.7±0.01	139.5±12.4	757.9±34.2	367.1±23.8	361.6±27.5
6	8.9±0.03	182.3±15.7	896.3±15.7	428.1±20.1	401.5±28.3
7	9.1±0.04	149.9±14.6	905.8±19.0	383.0±16.3	350.2±24.0

and remained steady for as long as the marker was eaten, the average concentration ranging between 7.6 and 7.7mg/gram of dried faeces. Similarly, the amount of barium excreted reached a plateau level, which fluctuated between 90.2% and 104.4% of the daily intake, on the third day (Table 14). As expected, on no single day did the excretion of barium exactly equal the intake; this is because of the slight variations in the amount of faeces excreted daily. However, over the period of feeding the marker, the total excretion of barium, as shown on table 15, quite closely approximated the intake, except for two pairs of rats. After reintroduction of unmarked food, both the concentration and the total amount of barium excreted daily in the faeces declined sharply. On the third day of refeeding the unmarked food there was virtually no barium in the faeces, concentration being, on average, 0.05mg/gram of dried stools, with a range of 0.0 to 0.1mg/g. On the fourth day there was no detectable barium in the faeces, apart from the baseline amount of  $60 \pm 5$  ppm.

#### Electrolytes in rat faeces.

The concentration and excretion in faeces, of calcium, chloride, potassium, magnesium and sodium were determined from samples collected during the first week of feeding unmarked pellet food. Results are presented in table 16. Because of variations in the amount of stools excreted daily, the day to day amount of any electrolyte excreted was variable.

#### Discussion.

In this study, rats fed on a diet containing 0.20% barium sulphate (as barium) w/w for seven days showed no signs of ill health. The

concentration and excretion of barium in the faeces rose rapidly reaching a plateau level on the third day of feeding the marker. A steady state was observed in which the excretion of barium averaged 95.9% of the mean daily intake. These results are comparable with those described by Davignon et al., 1968, in which 28 patients who had attained an ideal steady state with Chromic Oxide, excreted in faeces, on average, 90% of the daily chromium intake.

Since there is lack of information regarding recoveries on Barium Sulphate in the rat or other related animals, results obtained in this study are compared with observations made in human studies. Figueroa et al., 1968, orally administered 0.5g BaSO<sub>4</sub> daily to five patients and determined barium sulphate by the gravimetric method; their recoveries on the fed marker, measured from faeces collected over five days, averaged 103 ± 2.1% of the total intake, the range being 97.7 to 104.0%. In a similar balance study and applying the gravimetric method to analyse the content of barium sulphate in faeces, Dick (1967) obtained in 16 patients recoveries ranging between 90% and 104%, with a mean of 97% of the total intake. Scintillation counting was used by Boender and Verloop (1969) to determine recoveries in faeces, of an oral dose of <sup>131</sup>BaSO<sub>4</sub>, in normal persons and in patients with haemosiderosis; a range of 93 to 104% of the intake was recovered. In the present study, recoveries on fed barium ranged from 81.0 to 106.2% with a mean of 96.0% of the total intake. These results are comparable to those observed in human studies and show that, in the rat, barium sulphate eaten with the diet is almost completely recoverable from the faeces.

Furthermore, the present results indicate that measurement of barium sulphate (as barium) in the rat diet and faeces, provided results similar to those obtained when barium sulphate added to human faeces is

measured by chemical analysis. Figueroa et al. (1968) recovered 96.8 to 103.6% with a mean of 100.8% of barium sulphate added to human faeces; analysis was by the gravimetric method. Estimating  $\text{BaSO}_4$  by emission flame photometry, Dick (1969) obtained 98.2 to 101.7% recoveries from human stools, with a mean of 97.7% of the added marker. In the present study recoveries on added barium by the XRF method was 99.6%, 100.8% and 100.7%. However, as it has been pointed out already, the number of observations made on recovery of added barium in this case are too few to allow definitive conclusions to be made. Further observations could not be made because there was not enough financial support to allow continuation of experiments.

On the other hand, results gained in the present study indicate that barium sulphate is a satisfactory inert-marker for digestive studies in the rat. And it appears that XRF can be used as a rapid method of determining barium in biological material similar to rat faeces and rodent diets.

---



BIBLIOGRAPHY.

- ABERCROMBIE, M. (1946): Estimation of nuclear population from microtome sections. Anat. Rec. 94: 239-246.
- ADDIS, T., MAYERS, B.A. and OLIVER, J. (1924): The regulation of renal activity. IX. The effect of Unilateral nephrectomy on the function and structure of the remaining kidney. Arch. Intern. Med. 34: 243
- ADDIS, T. (1932): Hypertrophy of the gastrointestinal tract and high residual diets. Amer. J. Physiol. 99: 417-423.
- ADOLPH, E.F. (1947): Urge to eat and drink in rats. Amer. J. Physiol. 151: 110
- ALTHAUSEN, T.L., DOIG, R.K., UYEYAMA, K. and WEIDEN, S. (1950): Digestion and absorption after massive resection of the small intestine. Gastroenterology 16: 126-138
- ALTMANN, G.G. and ENESCO, M. (1967): Cell number as a measure of distribution and renewal of epithelial cells in the rat small intestine. Amer. J. Anat. 121: 319-336.
- ALTMANN, G.G. and LEBLOND, C.P. (1970): Factors influencing villus size in small intestine of adult rats as revealed by transposition of intestinal segments. Amer. J. Anat. 127: 15-36.
- ALTMANN, G.G. (1971): Influence of bile and pancreatic secretions on the size of intestinal villi in the rat. Amer. J. Anat. 132: 167-178.
- ALTMANN, G.G. (1972): Influence of starvation and refeeding on mucosal size and epithelial renewal in the rat small intestine. Amer. J. Anat. 133: 391.
- ALTMANN, G.G. (1974): in Proceedings of an International Conference on the Anatomy, Physiology, and Biochemistry of Intestinal Adaptation; pp 75-85; 1974 edition, by R.H. Dowling and E.O. Riecken.
- AMON, H.V. and PHILLIPS, S.F. (1972): Fatty acids inhibit water absorption in man. Gastroenterology 62: 717
- ANAND, B.K. and BROBECK, J.R. (1951a): Hypothalamic control of food intake in rats and cats. Yale J. Biol. and Med. 24: 123-140.
- ANAND, B.K. and BROBECK, J.R. (1951b): Localization of a "feeding centre" in the hypothalamus of the rat and the cat. Proc. Soc. Exper. Biol. Med. 77: 323-324.
- ANAND, B.K., DUA, S. and SHOENBERG, K. (1955): Hypothalamic control of food intake in cats and monkeys. J. Physiol. (Lond.) 127: 143-152.

- ANAND, B.K., SUBBERWAL, V., MANCHANDA, S.K. and SINGH, B. (1961): Glucoreceptor mechanism in the hypothalamic feeding centre. Ird. J. Med. Res. 49: 717-724.
- ANAND, B.K., CHHINA, G.S., SHARMA, K.N., DUA, S. and SINGH, B. (1964): Activity of single neurons in the hypothalamic feeding centre: effect of glucose. Amer. J. Physiol. 207: 1146-1154.
- ANNISON, E.F., HILL, K.J. and KENWORTHY, R. (1968): Volatile fatty acids in the digestive tract of the fowl. Brit. J. Nutr. 22: 207-216
- ANDERSON, R.R. and TURNER, C.W. (1963): Food consumption during lactation and involution in Sprague-Dawley Rolfsmeyer rats. Proc. Soc. Exper. Biol. Med. 113: 334-336.
- BALLARD, F.J., KROMFIELD, D.S. and HANSON, R.W. (1969): Gluconeogenesis and lipogenesis in tissues from ruminants and non ruminant animals. Fed. Proc. 28: 218-231.
- BANCROFT, J.R., McANALLY, R.A. and PHILLIPSON, A.T. (1953): Absorption of volatile fatty acids from the alimentary tract of sheep and other animals. J. Exper. Biol. 20: 120
- BARBER, R.S., BRAUDE, R., KON, S.K. and MITCHEL, K.L. (1953): Antibiotics in the diet of the fattening pig. Brit. J. Nutr. 7: 306
- BARKER, R.D. and SEARLE, G.W. (1960): Bile salt absorption at various levels of rat small intestine. Proc. Soc. Exper. Biol. Med. 105: 521-523.
- BARKER, S.J., MATHAN, V.I. and CHERIAN, V. (1963): The nature of the villi in the small intestine of the rat. Lancet 1: 860
- BARNES, R.H., FIALA, G., MCGEHEE, B. and BROWN, A. (1957): Prevention of coprophagy in the rat. J. Nutr. 63: 489
- BARNES, R.H. and FIALA, G. (1958): Effect of the prevention of coprophagy in the rat: ii - Vitamin B<sub>12</sub> requirement. J. Nutr. 65: 103-114.
- BARNES, R.H., KWONG, E. and FIALA, G. (1958): Effects of the prevention of coprophagy in the rat: iii - Digestibility of protein and fat. J. Nutr. 65: 251-258.
- BARNES, R.H., KWONG, E. and FIALA, G. (1959): Effects of the prevention of coprophagy in the rat: iv - Biotin. J. Nutr. 67: 599-610.
- BARNES, R.H. and FIALA, G. (1959): Effects of the prevention of coprophagy in the rat: vi - Vitamin K. J. Nutr. 68: 603-614.
- BARNES, R.H., TUTHILL, S., KWONG, E. and FIALA, G. (1959): Effects of the prevention of coprophagy in the rat: v - Essential fatty acids. J. Nutr. 68: 121-129.

- BATES, M.W., MAYER, J. and NAUSS, S. (1955): Fat metabolism in three forms of experimental obesity: Fatty acid turnover. Amer. J. Physiol. 180: 309
- BERGEIM, O. (1926): A method for the study of food utilization or digestibility. J. Biol. Chem. 70(1): 29-33.
- BERTALANFFY, F.D. (1960): Mitotic rates and renewal times of the digestive tract epithelia in the rat. Acta Anat. 40: 130-147.
- BERTALANFFY, F.D. and NAGY, K.P. (1961): Mitotic activity and renewal rates of the epithelial cells of the human duodenum. Acta Anat. 45: 362-370.
- BEST, R.R. (1948): Anastomosis of ileum to the lower part of the rectum and anus. Arch. Surg. 57: 276-285.
- BEST, R.R. (1952): Evaluation of ileoproctostomy to avoid ileostomy in various colon lesions. J. Amer. Med. Assoc. 150: 637-642.
- BOENDER, C.A. and VERLOOP, M.C. (1969): Iron absorption, iron loss and iron retention in man: Studies after oral administration of a tracer dose of  $^{59}\text{FeSO}_4$  and  $^{131}\text{BaSO}_4$ . Brit. J. Haematol. 17:45-58
- BORGSTOM, B., LUDH, G. and HOFMANN, A. (1963): The site of absorption of conjugated bile salts in man. Gastroent. 45: 229-238.
- BOYD, E.D. and ABEL, M. (1966): Acute toxicity of barium sulphate administered intragastrically. Canad. Med. Assoc. J. 94: 849-853.
- BRIGH-ASSARE, P. and BINDER, H.J. (1973): Stimulation of colonic secretion of water and electrolytes by hydroxy fatty acids. Gastroenterology 64: 81-88.
- BROOKS, C. McC., LAMBERT, E.F. and BARD, P. (1942): Experimental production of obesity in the monkey. Fed. Proc. 1.11
- BROBECK, J.R. (1948): Food intake as mechanism of temperature regulation. Yale. J. Biol. and Med. 20: 545-552.
- BROBECK, J.R., TEPPERMAN, J. and LONG, C.N.H. (1943): Experimental hypothalamic hyperphagia in albino rats. Yale. J. Biol. & Med. 15: 831
- BROBECK, J.R. (1955): Neural regulation of food intake. Ann. N. Y. Acad. Sci. 63: 44-55.
- BUCHANAN, J.M., HASTINGS, A.B. and NESBETT, F.B. (1943): The role of carbohydrate-labelled acetic, proprionic and butyric acids in liver glycogen formation. J. Biol. Chem. 150: 413.
- BUCHER, N.L.R., SWAFFIELD, M.N. and Di TROIA, J.F. (1964): Influence of age upon incorporation of thymidine -  $2\text{-C}^{14}$  into DNA of regenerating rat liver. Cancer Res. 24: 509-512.

- BUCHER, N.L.R., and SWAFFIELD, M.N. (1964): The rate of incorporation of labelled thymidine into the deoxyribonucleic acid of regenerating rat liver in relation to the amount of liver excised. Cancer Res. 24: 1611-1625.
- BUCHER, N.L.R. (1967): Experimental aspects of hepatic regeneration. New Engl. J. Med. 277: 686-738.
- BULLOUGH, W.S. (1965): Mitotic and functional homeostasis: A speculative review. Cancer Res. 25: 1683-1727.
- CAIRNIE, A.B., LAMERTON, L.F. and STEEL, G.G. (1965): Cell proliferation studies in the intestinal epithelium of the rat. Exper. Cell Res. 39: 528-538.
- CAMPBELL, R.M. and FELL, B.F. (1964): Gastrointestinal hypertrophy in the lactating rat and its relation to the food intake. J. Physiol. (Lond.). 171: 90-97.
- CLARKE, R. (1968): A comparative analysis of methods of estimating the size of cell population from microtome sections. J. Roy. Microscop. Soc. 88: 189-203.
- CLARKE, R.M. (1974): in Proceedings of an International Conference on the Anatomy, Physiology and Biochemistry of Intestinal Adaptation, pp 11-16; 1974 edition, by R.H. Dowling and E.O. Riecken.
- CLATWORTHY, H.W., Jr., SALEEBY, R. and LOVINGOOD, C. (1952): Extensive small bowel resection in young dogs: Effect on growth and development. Surgery 32: 341-349.
- CLAWSON, A.J., REID, J.T., SHEFFY, B.E. and WILLMAN, J.P. (1955): Use of chromium oxide in digestion studies with swine. J. Anim. Sci. 14: 700-714.
- COATES, M.E., HENRY, K.M., KON, S.K., MAWSON, E.H., STAINER, J.E. and THOMSON, S.Y. (1946): Sulphonamides and Potato starch refection in the rat. Nature 157: 262-263.
- COLE, H.H. and HART, G.H. (1938): The effect of pregnancy and lactation on growth in the rat. Amer. J. Physiol. 123: 589-597.
- COLEMAN, S.T. and ECKERT, C. (1955): Preservation of rectum in familial polyposis of the colon and rectum. Arch. Surg. 73: 635-644.
- COUCH, J.R., GERMAN, H.L., KNIGHT, D.R., SPARKS, P. and PEARSON, P.B. (1950): Importance of the caecum in Intestinal synthesis in the mature domestic fowl. Poultry Sci. 29: 52-58.
- CRAFT, I.L. (1970): The influence of pregnancy and lactation on the morphology and absorptive capacity of the rat small intestine. Clin. Sci. 38: 287-295.

- CRANE, J.W. and HENDERSON, V.E. (1924): The sensitivity of the small intestine at different levels to internal pressure. Amer. J. Physiol. 70: 22-25.
- CRAWFORD, N. and BROOKE, B.N. (1953): Ileostomy chemistry. Lancet 1: 864-867.
- CREAN, G.P., MARSHALL, M.W. and RUMSEY, R.D.E. (1969): Parietal cell hyperplasia induced by administration of pentagastrin (ICI 50,120) to rats. Gastroenterology 57: 147-155.
- CREGAN, J. and HAYWARD, N.J. (1953): The bacterial content of the healthy human small intestine. Brit. Med. J. 1: 1356-1359.
- CRIPPS, A.W. and WILLIAMS, V.J. (1975): The effect of pregnancy and lactation on food intake, gastrointestinal anatomy and absorptive capacity of the small intestine in rats. Brit. J. Nutr. 33: 17-32.
- CRIPPS, A.W. (1972): Gastrointestinal function during pregnancy and lactation in the rat. B.Sc. Thesis, University of England Armidale, New South Wales, Australia; cited by Cripps and Williams, in Brit. J. Nutr. 33: 17; 1975.
- CUMMINGS, J.H., JAMES, W.P. and WIGGINS, H.S. (1973): Role of the colon in ileal resection diarrhoea. Lancet 1: 344-347.
- CUTHBERTSON, D.P. (1932): Disturbance of metabolism produced by injury to the limbs. Quart. J. Med. 25: 233
- CUTHBERTSON, D.P. (1959): Protein metabolism in relation to energy need: Symposium on metabolism after trauma. Metabolism 8: 787-807.
- DAHLQVIST, A. (1967): Localization of small intestinal disaccharidases. Amer. J. Clin. Nutr. 20: 81-87.
- DAVENPORT, H.W. (1971): Physiology of the digestive tract, 3rd edition; Year Book Medical Publishers, Chicago.
- DAVIGNON, J.R., SIMMONDS, W.J. and AHRENS, E.H. (1968): Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of colonic function. J. Clin. Invest. 47(1): 127-138.
- DAVSON, M.G. and ENGLETON, J.A. (1968): Principles of Human Physiology, 4th edition, Churchill Ltd.
- DAY, H.G., WAKIN, K.G., KRIDER, M.M. and O'BANION, E.E. (1943): Effects of caecectomy, succinylsulfathiazole and p-Aminobenzoic acid on vitamin K synthesis in the intestinal tract of rats. J. Nutr. 26: 585-600.
- DEMARNE, P. (1972): cited in Nutr. Abstr. & Rev. 43: 118; 1973

- DEVROEDE, G.J. and PHILLIPS, S.F. (1970): Failure of the human rectum to absorb water and electrolytes. Gut. 11: 438-442.
- DICK, M. (1967): Use of barium sulphate as a continuous marker for faeces. J. Clin. Path. 20: 216-218.
- DICK, M. (1969): Estimation of barium sulphate in faeces by emission flame photometry. J. Clin. Path. 22: 378
- DOLE, V.P., DAHL, L.K., GOTZIAS, G.C., EDER, H.A. and KREBS, M.E. (1950): Dietary treatment of hypertension; clinical and metabolic studies of patients on the rice-fruit diet. J. Clin. Inv. 29: 118
- DONALDSON, H. (1923): On changes in the relative weights of viscera and other organs from birth to maturity in albino rats. Amer. J. Physiol. 67: 1-21.
- DONALDSON, H.H. (1924): The Rat: Data and reference tables for the albino rat; page 179. Philadelphia.
- DORNFELD, E.J., SALTER, D.W. and SCHEFFE, H. (1942): A method for accurate determination of volume and cell number in small organs. Anat. Rec. 82: 255-259.
- DOWLING, R.H. (1967): Compensatory mechanism in the small intestine. Brit. Med. Bull. 23: 275-278.
- DOWLING, R.H. and BOOTH, C.C. (1967): Structure and functional changes following small intestinal resection in the rat. Clin. Sci. 32: 139-149.
- DOWLING, R.H., RIECKEN, E.O., LAWS, J.W. and BOOTH, C.C. (1967): Intestinal response to high bulk feeding in the rat. Clin. Sci. 32: 1-9.
- DOZOIS, R.R. and KELLY, K.A. (1970): Effect of gastrin pentapeptide on gastric emptying of liquids. Surg. Forum 21: 293-295.
- DRASAR, B.S. and SHINER, M. (1969): Bacterial flora of the small intestine in patients with gastrointestinal disorders. Gut. 10: 812-819.
- DRASER, B.S., HILL, M.J. and SHINER, M. (1966): The deconjugation of bile salts by human intestinal bacteria. Lancet 1: 1237-1238.
- EDEN, A. (1953): Coprophagy in the rabbit. Nature 145: 36; 628.
- ELIAS, E. and DOWLING, R.H. (1974): in Proceedings of an International Conference on the Anatomy, Physiology and Biochemistry of Intestinal Adaptation; pp 94-97; edited by R.H. Dowling and E.O. Riecken, 1974.
- ELMA, C.J., PUPNAM, P.A. and DAVIS, R.E. (1959): Faecal excretion pattern of chromic oxide administered to Hereford heifers in a completely pelleted form. J. Anim. Sci. 18: 718-724.

- ELSDEN, S.R., HITCHCOCK, W.S., MARSHALL, R.A. and PHILLIPSON, A.T. (1946): Volatile acids in the digesta of ruminants and other animals. J. Exper. Biol. 22: 191-202.
- EWING, G.W. (1969): Instrumental Methods of Chemical Analysis, 3rd edit., McGraw-Hill; Kogakusha Ltd., Tokyo.
- FABRY, P. and KUJALOVA, V. (1960): Enhanced growth of the small intestine in rats as a result of adaptation to intermittent starvation. Acta Anat. 43: 264-271.
- FABRY, P. and KUJALOVA, V. (1960 b): cited in Feeding Pattern and Nutritional Adaptations, p 41, 1969 edition, by Fabry, P., translated by K. Osancova, London: Butterworths.
- FARREL, J.I. and IVY, A.C. (1926): Studies on the motility of the transplanted gastric pouch. Amer. J. Physiol. 76: 227-228.
- FELL, B.F., SMITH, K.A. and CAMPBELL, R.M. (1963): Hypertrophic and hyperplastic changes in the alimentary canal of the lactating rat. J. Path. Bact. 85: 179-188.
- FELL, B.F. (1972): cited by B.A. ROLLS, Brit. J. Nutr. 33 (1): 1; 1975.
- FIGUEROA, W.G., JORDAN, T. and BASSETT, S.H. (1968): Use of barium sulphate as unabsorbable faecal marker. Amer. J. Clin. Nutr. 21: 1239-1245.
- FISCHER, J.E. (1957): Effects of feeding diets containing lactose, agar, cellulose, raw potato starch or arabinose on the dry weight of cleaned gastrointestinal organs in the rat. Amer. J. Physiol. 188: 550
- FISHBACK, F.C. (1929): A morphologic study of regeneration of the liver after partial removal. Arch. Pathol. 7: 955-977.
- FISHER, R.B. and PARSONS, D.S. (1950): The gradient of mucosal surface area in the small intestine of the rat. J. Anat. 84: 272-282.
- FORREL, M., OTTE, M., KOHL, H.J., LEHNERT, P. and STAHLHEBER, H.P. (1972): Bile salts: Pancreatic secretagogues. Gastroenterology 63: 711-712.
- FOURNIER, P. and DUPIS, Y. (1953): Le sort des composés phosphorés de la ration dans les diverses parties du tube digestif du rat adulte. J. Physiologie (Paris). 45: 451-461.
- FOURNIER, P. (1950): cited by Kotb and Luckey, Nutr. Abstr. & Rev. 42: 813, 1972.
- FOWLER, D.I., COOKE, W.T., BROOKE, B.N. and COX, E.V. (1959): Ileostomy and electrolyte excretion. Amer. J. Dig. Dis. 4: 710-720.
- GALLAGHER, N.D., HARRISON, D.D. and SKYRING, A.P. (1962): Fluid and electrolyte disturbances in patients with long established ileostomies. Gut. 3: 219-223.

- GALLUP, W.D. (1928): A note on the determination of digestibility of protein by Bergeim method. J. Biol. Chem. 81 (2): 321-324.
- GAZET, J.C. and KOPP, J. (1964): The Surgical significance of the ileocaecal valve. Surgery 56: 565-573.
- GAZET, J.C.: quoted by Gazet and Kopp, Surgery 56: 565, 1964.
- GARBBUTT, J.T., LACK, L. and TYOR, M.P. (1971): The enterohepatic circulation of bile salts in gastrointestinal disorders. Amer. J. Med. 51: 627-636.
- GILLER, J. and PHILLIP, S.F. (1970): Colonic absorption of water and electrolytes: A comparison of 24-hour ileal contents and faeces. Gastroenterology 58: 951-952.
- GLEESON, M.H., DOWLING, R.H. and PETERS, T.J. (1972)<sup>b</sup>: Mucosal enzymes after small bowel bypass in the rat. Clin. Sci. 43: 743-757.
- GLEESON, M.H., CULLEN, J. and DOWLING, R.H. (1972a): Intestinal structure and function after small bowel bypass in the rat. Clin. Sci. 43: 731-742.
- GOLDMAN, L.I., DORN, B., WEINBERGER, M., BRALOW, S.P. and GRUENSTEIN, M. (1972): The small intestine and gastric secretion in the rat. Arch. Surg. 104: 73-75.
- GRACEY, M. (1971): Intestinal absorption in the contaminated small-bowel-syndrome. Gut. 12: 403-410.
- GRIFFITH, W.H. (1935): Studies on growth: iii - B and G avitaminosis in caeectomized rats. J. Nutr. 10: 667-674.
- GROSSMAN, M.I. (1955): Integration of current views on the regulation of hunger and appetite. Ann. N. Y. Acad. Sci. 63: 76-91.
- GROSSMAN, M.I., CUMMINS, G.M. and IVY, A.C. (1947): The effect of insulin on food intake after vagotomy and sympathectomy. Amer. J. Physiol. 149: 100-102.
- GUERRANT, N.B., DUTCHER, R.A. and TOMEY, L.F. (1935): The effect of the type of carbohydrate on the synthesis of B vitamins in the digestive tract of the rat. J. Biol. Chem. 110: 233-243.
- GUERRANT, N.B. (1955): quoted by Mickelsen, O. in Vitamins & Hormones 14: 3; 1956.
- HAGEN, P. and ROBINSON, K.W. (1953): The production of volatile fatty acids in the intestine of the guinea-pig. Austr. J. Exper. Biol. Med. Sci. 31: 99-103.



- HAGIHARA, P.F. and GRIFFEN, O. (1972): Physiology of the colon and rectum. Surg. Clin. North Amer. 52: 797-805.
- HALL, R.J. and CREAMER, B. (1974): Hyperphagia in intestinal disease. Gut. 25 (15): 858-861.
- HAMMER, J.M., SEAY, H., JOHNSTON, R.L., HILL, E.J., PRUST, F.H. and CAMPBELL, R.J. (1959): Antiperistaltic bowel segment on intestinal emptying time. Arch. Surg. 79: 537-541.
- HARLOW, H.F. (1932): Food intake by individually housed rats. J. Genet. Psychol. 41: 211.
- HARRIES, J.T. and SLADEN, G.E. (1972): The effects of different bile salts on the absorption of fluids, electrolytes and monosaccharides in the small intestine of the rat in vivo. Gut. 13: 596-603.
- HAYSLETT, J.P., KASHGARIAN, M. and EPSTEIN, F.H. (1968): Functional correlates of compensatory renal hypertrophy. J. Clin. Invest. 47: 774.
- HERNNING, S.J. and HIRD, F.J.D. (1972): Diurnal variations in the concentrations of volatile fatty acids in the alimentary tracts of wild rabbits. Brit. J. Nutr. 27: 57-64.
- HEROUX, O. and GRIDGEMAN, N.T. (1958): The effect of cold acclimation on the size of organs and tissue of the rat, with special reference to modes of expression of results. Canad. J. Biochem. and Physiol. 36: 209
- HETHERINGTON, A.W. and RANSON, S.W. (1942): The spontaneous activity and food intake in rats with hypothalamic lesions. Amer. J. Physiol. 136: 609
- HETHERINGTON, A.W. and RANSON, S.W. (1940): Hypothalamic lesions and adiposity in the rat. Anat. Rec. 78: 148-172.
- HILL, J. and DRASAR, B.S. (1968): Degradation of bile salts by human intestinal bacteria. Gut. 9: 22-27.
- HOFMANN, A.F. (1967): The syndrome of ileal disease and the broken enterohepatic circulation of bile acids. Gastroenterology 52: 752-757.
- HOFMANN, A.F. and POLEY, J.R. (1972): Role of bile acid malabsorption in the pathogenesis of diarrhoea and steatorrhoea in patients with ileal resection. Gastroenterology 62: 918-934.

- HOLECKOVA, E. and FABRY, P. (1959): Hyperphagia and gastric hypertrophy in rats adapted to intermittent starvation. Brit. J. Nutr. 13:260-265.
- HOLT, P.R. (1964): Intestinal absorption of bile acids in the rat. Amer. J. Physiol. 207: 1-7.
- HOOPER, C.E.S. (1961): Use of colchicine for the measurement of mitotic rate in intestinal epithelium. Amer. J. Anat. 108: 231-244.
- HOOVER, W.H., MANNING, C.L. and SHEERIN, H.E. (1968): Observation pertaining to digestion in the golden hamster. J. Anim. Sci. 27: 1512.
- HUANG, T.C., ULRICH, H.E. and McCAY, C.M. (1954). Antibiotics, growth, food utilization and the use of chromic oxide in studies with rabbits. J. Nutr. 54: 621-630.
- HUGO, V., ABBOTT, A.G., WILLIAMS, R.J., ROSEN, H. and ORLOFF, M. (1971): Nature of the gastric hypersecretion associated with intestinal exclusion. Surg. Forum. 22: 299-300.
- HUGHES, E.S.R. and BENNETT, R.C. (1972): Caecal pull-through operation for distal ulcerative colitis. Austr. NZ. Surg. 42: 26-30.
- HUNT, J.N. and KNOX, M.T. (1972): The slowing of gastric emptying by acids. J. Physiol. (Lond.). 222: 187-208.
- IRWIN, M.I. and CRAMPTON, E.W. (1951): The use of chromic oxide as an index material in digestion trials with human subjects. J. Nutr. 43: 77-85.
- JAMES, A.T., WEBB, J.P.W. and KELLOCH, T.D. (1961): The occurrence of unusual fatty acids in faecal lipids from human being with normal and abnormal fat absorption. Biochem. J. 78: 333-339.
- JANOWITZ, H.D. and GROSSMAN, M.I. (1951): Effects of prefeeding, alcohol, and bitters on food intake of dogs. Amer. J. Physiol. 164: 182-186.
- JANOWITZ, H.D. and GROSSMAN, M.I. (1949): Some factors affecting the food intake of normal dogs and dogs with esophagostomy and gastric fistula. Amer. J. Physiol. 159: 143-148.
- JENKINS, P. and De VRIES, M. (1967): Practical X-ray Spectrometry.
- JERVIS, E.L. and LEVIN, R.S. (1966): Anatomic adaptation of the alimentary tract of the rat to hyperphagia of chronic alloxan-diabetes. Nature (London). 210: 391-393.
- JOHNASSON, K.R., PETERSON, C.E. and DICK, E.C. (1953). Effects of dietary Auriomycin upon the intestinal synthesis of B<sub>12</sub> in the rat. J. Nutr. 49: 135-152.
- JOHNASSON, K.R. and NYLANDER, G. (1968): Analysis of the ileocaecal emptying mechanism in the rat. Acta. Chir. Scand. 134: 296-302.

- JOHNSON, J.L. and McBEE, R.H. (1967): The porcupine faecal fermentation. J. Nutr. 91: 540-546.
- KAIMO, C. (1969): Importance of ileocaecal junction in patients with small bowel resection. Gastroenterology. 57: 606-607.
- KALSER, M.H., ROTH, J.L.A., TUMEN, H. and JOHNSON, J.A. (1960): Relation of small bowel resection to nutrition in man. Gastroenterology. 38: 605-615.
- KAMEOKA, K., TAKAHASHI, S. and MORIMOTO, H. (1956): Variation of the excretion of Chromic oxide in ruminants. J. Dairy Sci.: 39: 462-467.
- KANAGHINIS, T., LUBRAN, M. and COGHILL, N.F. (1963): The composition of ileostomy fluid in man. Gut 4: 322-338.
- KANE, E.A., JACOBSON, W.C. and MOORE, L.A. (1950): A comparison of techniques used in digestibility studies with dairy cattle. J. Nutr. 41: 583-596.
- KELLOCK, T.D., PEARSON, J.R., RUSSEL, R.I., WALKER, J.G. and WIGGINS, H.S. (1969): The incidence and clinical significance of faecal hydroxy fatty acids. Gut 10: 1055
- KERSHOW, T.G., NEAME, K.D. and WISEMAN, G. (1960): The effect of semi-starvation on absorption by the rat small intestine in vivo and in vitro. J. Physiol. (Lond.). 152: 182-189.
- KIM, Y.S., SPRITZ, N., BLUM, M., TREZ, J. and SHERLOCK, P. (1966): The role of altered bile acid metabolism in the steatorrhoea of experimental blind loop in dogs. J. Clin. Invest. 45: 956.
- KIM, Y.S. and SPRITZ, N. (1968): Hydroxy fatty acid excretion in faeces. New Engl. J. Med. 279: 1424-1426.
- KNUDTSON, K.P. PRIEST, R.E., JACKLIN, A.J. and JESSEPH, J.E. (1962): Effect of partial resection in mammalian small intestine. Lab. Invest. 11: 433-437.
- KOTB, A.R. and LUCKEY, T.D. (1972): Markers in nutrition. Nutr. Abstr. & Rev. 42: 813-845.
- KRAMER, P., KEARNEY, M.M. and INGELFINGER, F.J. (1962): The effect of specific foods and water loading on ileal excreta of ileostomized human subjects. Gastroenterology. 42: 535-545.
- KRAMER, P. (1966): The effect of varying sodium loads on the ileal excretion of human ileostomized subjects. J. Clin. Invest. 45: 1710.
- KRAUS, R.M. and MAYER, J. (1965): Influence of protein and aminoacids on food intake in the rat. Amer. J. Physiol. 209: 479-483.

- KUJALOVA, V. and FABRY, P. (1960): Intestinal absorption of glucose, fat and amino acids in rats adapted to intermittent starvation. Physiol. Bohem. 9: 35.
- KULWICH, R., STRIGILA, L. and PEARSON, J. (1953): The effect of coprophagy on the excretion of B vitamins in the rabbit. J. Nutr. 49: 639-645.
- LAMBERT, R. (1965): Surgery of the Digestive System in the Rat: Charles C. Thomas: Publisher. Springfield, Illinois, U.S.A.,
- LEBENTHAL, E., SUNSHINE, P. and KRETCHMER, N. (1972): Effect of carbohydrate and corticosteroids on activity of  $\alpha$ -glucosidase in the intestine of infant rats. J. Clin. Invest. 51: 1244-1250.
- LEBLOND, C.P. and STEVENS, C.E. (1948): The constant renewal of intestinal epithelium in the albino rat. Anat. Rec. 100: 357-371.
- LEBLOND, C.P., STEVENS, C.E. and BOGOROCH, R. (1948): Histological localization of newly formed Deoxyribonucleic acid. Sci. 108: 531-533.
- LEBLOND, C.P. and MESSIER, B. (1958): Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of Thymidin- $H^3$  into mice. Anat. Rec. 132: 247-260.
- LESHER, S. and BAUMAN, J. (1969): Cell kinetic studies of the intestinal epithelium: Maintenance of the intestinal epithelium in normal and irradiated animals. Cancer Instit. Monograph. 30: 185.
- LEVIN, R.S., GELFAND, M., HERKOVIC, T. and FLOCH, M.H. (1968): Bacterial flora of patients with total and partial colon resection. Gastroenterology. 54: 1253.
- LEVITAN, R., FORDTRAN, J.S., BURROW, B.A. and INGELFINGER, F.Z. (1962): Water and salt absorption in the human colon. J. Clin. Invest. 41: 1754-1759.
- LILLEHEI, R.C. and WANGENSTEEN, O.H. (1955): Bowel function after colectomy for cancer, polyps and diverticulitis. J. Amer. Med. Ass. 159: 163-170.
- LINDSTEDT, S. and SAMUELSON, B. (1963): Bile acids and steroids: LXXXii On the interconversion of cholic and deoxycholic acids in the rat. J. Biol. Chem. 234: 2026.
- LIPKIN, M., SHERLOCK, P. and BELL, B. (1963): Cell proliferation kinetics in the gastrointestinal tract of man. Gastroenterology. 45: 721-729.
- LOCKWOOD, J.S. and RANDALL, H.T. (1949): Gastrointestinal tract losses of electrolytes. Bull. N.Y. Acad. Med. 25: 228-243.

- LORAN, M.R. and ALTHAUSEN, T.L. (1958): Hypertrophy and changes in Cholinestrase activities of the intestine, erythrocytes and plasma after partial resection of the small intestine in the rat. Amer. J. Physiol. 193: 516-520.
- LORAN, M.R. and ALTHAUSEN, T.L. (1960): Cellular proliferation of the intestinal epithelium in the rat. J. Biophysic. Biochem. Cytol. 7: 667-671.
- LORAN, M.R. and CROCKER, T.T. (1963): Population dynamics of intestinal epithelium in the rat 2 months after partial resection of ileum. J. Cell. Biol. 19: 285-290.
- LOBBER, V., LIFSON, N. and WOOD, H.G. (1959): The position of fixed carbon in glucose from rat liver glycogen. J. Biol. Chem. 159: 475-489.
- LOUIS, M. and SLATTERY, J.R. (1964): Immediate complications following resection of the large intestine. Surg. Clin. N. Amer. 44(2): 397-410.
- LUTTON, C.L. and CHEVALLIER, F. (1973): Coprophagy in the white rat: Quantitative aspects and time-relation with food intake. J. Physiol. (Paris). 66: 219-228.
- LYTTON, B., SCHITT, M.Jr. and BLOOM, N.A. (1969): Compensatory renal growth: Evidence for tissue specific factor of renal origin. J. Urol. 101: 648-652.
- MacDONALD, R.A., ROGERS, A.E. and PECHET, G. (1962): Regeneration of the liver: Relation of regenerative response to size of partial hepatectomy. Lab. Invest. 11: 544.
- MacDONALD, W.C., TRIER, J.S. and EVERETT, N.B. (1964): Cell proliferation and migration in the stomach, duodenum and rectum of man: Radioautographic studies. Gastroenterology. 46: 105.
- MACHELLA, E. and GRIFFITH, J.Q. (1949): in The Rat in Laboratory Investigations pp 166-167; 2nd edition, by Farris and Griffith, J.Q., 1949.
- MacKINNON, A.M. (1973): Functional adaptation of vitamin B<sub>12</sub> absorption in the guinea-pig following small bowel resection. Amer. J. Dig. Dis. 18: 576-582.
- MARRABLE, A.W. (1962): The counting of cells and nuclei in microtome sections. Quart. J. Microscop. Sci. 103: 33-347.
- MARTRINDALE, W. (1972): The Extra Pharmacopoeia, 26th ed. London Pharm. Press.
- MAYER, J., FRENCH, R.G., ZIGHERA, C.F. and BARNET, R.J. (1955): Hypothalamic obesity in the mouse: production, description and metabolic characteristics. Amer. J. Physiol. 182: 75.

- MAYER, J. (1952): The Glucostatic theory of regulation of food intake and the problem of obesity. Bull. New Engl. Med. Cent. 14: 43.
- MAYER, J. and BATES, M.W. (1952): Blood glucose and food intake in normal and hypophysectomised, alloxan-treated rats. Amer. J. Physiol. 168: 812-819.
- MAYER, J. (1953): Genetic, traumatic and environmental factors in the etiology of obesity. Physiol. Rev. 33: 472-508.
- MAYER, J. (1955): Regulation of energy intake and body weight: the Glucostatic theory and the Lipostatic hypothesis. Ann. N.Y. Acad. Sci. 63: 15-41.
- MAYER, J. and MARSHALL, B. (1956): Specificity of gold thioglucose for ventromedial hypothalamic lesions and hyperphagia. Nature. 178: 1399
- McDONALD, D.G., STERN, J.A. and HAHN, W.W. (1963): Effects of differential housing and stress on diet selection, water intake and body weights in rats. J. Appl. Physiol. 18: 937-942.
- McGREGOR, M.A., PARSONS, H.T. and PETERSON, W.H. (1947): Biotin balance in the albino rat. J. Nutr. 33: 517-527.
- McMANUS, J.P.A. and ISSELBACHER, K.J. (1970): Effects of fasting versus feeding on rat small intestine. Gastroenterology. 59: 214-221.
- McMINN, R.M.H. (1954): The rate of renewal of intestinal epithelium in the cat. J. Anat. 88: 527-582.
- MEKHJIAN, H.S., PHILLIPS, S.F. and HOFMANN, A.F. (1968): Conjugated bile salts block water and electrolyte transport by human colon. Gastroenterology. 54: 1256.
- MEKHJIAN, H.S. and PHILLIPS, S.F. (1970): Perfusion of the canine colon with unconjugated bile acids. Gastroenterology. 59: 120-129.
- MEKHJIAN, H.S. and PHILLIPS, S.F. (1971): Colon secretion of water and electrolytes induced by bile acids: perfusion studies in man. J. Clin. Invest. 50: 1569-1577.
- MERCK INDEX (1968): Merck & Co. Rahwa, N.J. U.S.A.
- MICKELSEN, O. (1956): Intestinal synthesis of vitamins in the non-ruminant. Vitamins & Hormones. 14: 1-95.
- MOINUDDIN, J.F. and LEE, H.W. (1959): Possible association of dietary residues with growth of the large gut. Amer. J. Physiol. 197: 903-911.
- MOOLTEN, F.L. and BUCHER, N.L.R. (1967): Regeneration of rat liver: Humoral agents involved. Science. 158: 272-273.
- MOORE, J.H. (1957): Diurnal variations in the composition of the faeces of pigs on diets containing chromic oxide. Brit. J. Nutr. 11: 273-287.

- MUELLER, W.J. (1956): Feasibility of the chromic oxide and the lignin indicator methods for metabolism experiments with chickens. J. Nutr. 58 (1): 29-36
- NASR, H. (1950): The nutritional significance of microbial digestion of starch in the pig caecum. Brit. J. Nutr. 4: 5(v).
- NORMAN, A. and SJÖVALL, J. (1958): On the transformation of enterohepatic circulation of cholic acid in the rat. J. Biol. Chem. 233: 878-885.
- NYGAARD, K. (1967a): Resection of the small intestine in rats: (iii). Morphological changes in the intestinal tract. Acta. Chir. Scand. 133: 233-248.
- NYGAARD, K. (1967b): Resection of the small intestine in the rat: iv. Adaptation of gastrointestinal motility. Acta. Chir. Scand. 133: 407-416.
- NYGAARD, K. (1967c): Changes in the intestinal flora after resection and bypass operations on the small intestine in the rat. Acta. Chir. Scand. 133: 569-583.
- NYLANDER, G. and OLERUD, S. (1962): Intestinal adaptation following extensive resection in the rat. Acta. Chir. Scand. 123: 51-56.
- NYLANDER, G. (1963): Ileostomy: the effect on microvascular and intestinal villi of the rat. Acta. Chir. Scand. 125: 162-171.
- NORMAN, A. and GRUBB, R. (1955): Hydrolysis of conjugated bile acids by clostridia and enterococci. Acta. Path. Microbiol. Scand. 36: 537-547.
- NUGUID, T.P., BACON, H.E. and BOUTWELL, J. (1961): An investigation of the volume of output and chemical content of ileal discharge following total colectomy and ileostomy. Surg. Gynae & Obst. 133: 733.
- OGILVIE, W.H. (1931): Conservation of the ileocaecal sphincter in resection of the right half of the colon. Brit. J. Surg. 19: 8-24.
- PADYKULA, H.A. (1962): Recent functional interpretation of intestinal morphology. Fed. Proc. 21: 873.
- PARSONS, D.S. (1968): in the Handbook of Physiology; The Alimentary Canal, vol. 3, pp. 1177-1216; by W. Heidel; Amer. Physiological Soc.
- FERRY, P.M., MOK, H.Y.I. and DOWLING, R.H. (1974): in Proceedings of an International Conference on the Anatomy, Physiology and Biochemistry of Intestinal Adaptation, pp. 69-74. ed. by R.H. Dowling and E.O. Riecken, 1974.
- PHILLIPS, S.F. (1969): Absorption and secretion by the human colon. Gastroenterology. 56: 966-970.

- PHILLIPS, S.F. (1972): Diarrhoea: A current view of the Pathophysiology. Gastroenterology. 63: 495-518.
- PILLING, G.P. and CRESSON, S.L. (1957): Massive resection of the small intestine in the neonatal period. Paediatrics. 19: 940-948.
- PLAYOUST, M.R., LACK, L. and WEINER, I.M. (1965): Effect of intestinal resection on bile salt absorption in dogs. Amer. J. Physiol. 208: 363.
- PLUM, C.M. (1951): Experimental Macrocytic Anaemia in the rat. Le Sang. 21: 437-451.
- POOL, L.J., LEW, W. and ADDIS, T. (1939): Protein anabolism of organs and tissues during pregnancy and lactation. J. Biol Chem. 128: 69-77.
- PORUS, R.L. (1965): Epithelial hyperplasia following massive small bowel resection in man. Gastroenterology. 48: 753-757.
- POTTER, B., SAKAI, T., HARRAH, J. and HOLIDAY, M.A. (1969): Renal function and structure within 18 hours following uninephrectomy. Clin. Res. 17: 169.
- RAVITCH, M.M. (1947): Anal ileostomy with preservation of the sphincter in dogs. Surg. Gyna. & Obst. 84: 1095-1099.
- RAVITCH, M.M. (1948): Anal ileostomy with sphincter preservation in patients requiring total colectomy for benign conditions. Surgery. 24: 170.
- ROLLS, B.A. (1975): Dipeptidase activity in the small intestinal mucosa during pregnancy and lactation in the rat. Brit. J. Nutr. 33: 1-9.
- ROSE, G.A. (1964): Experience with the use of interrupted carmine red and continuous chromic sesquioxide marking of human faeces with reference to calcium, phosphorus, and magnesium. Gut. 5: 274-279.
- ROSI, P. and CAHILL, W.J. (1962): Subtotal colectomy with caecorectal anastomosis for multiple adenomas of the colon. Amer. J. Surg. 103: 75-80.
- RIECKEN, E.O., DOWLING, R.H., BOOTH, C.C. and PEARSE, A.G.E. (1965): Enzymol. Biol. Clin. 5: 231.
- RICHARDSON, J.D. (1970): Importance of the ileocaecal valve in intestinal absorption. Texas Rep. Biol. Med. 28: 408.
- RICHARDSON, J.D. and GRIFFEN, W.O. (1972): Ileocaecal valve substitutes as bacteriologic barriers. Amer. J. Surg. 123: 149-153.



- RUBINSTEIN, R., HOWARD, A.V. and WRONG, O.M. (1969): In vivo dialysis of faeces as a method of stool analysis: iv. The organic anion component. Clin. Sci. 37: 549-564.
- RUDERMAN, R.L. and KAMEL, I. (1970): The effect of intestinal resection on gastric acidity in the rat. Surg. Forum 21: 312-313.
- SAMUEL, P., SAYPOL, G., MEILMAN, E., MOSBACH, E.H. and CHAFIZADEH, M. (1968): Absorption of bile acids from the large intestine in man. J. Clin. Invest. 47: 2070.
- SEIFE, B. (1962): Radioactive inert-indicator method for intestinal absorption utilizing differential counting. J. Lab. & Clin. Med. 59: 513-519.
- SCHURCH, A.F., LLOYD, L.E. and CRAMPTON, E.W. (1950): The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 41: 629-636.
- SCHWEIGERT, B.S., McITIRE, J.M., HENDERSON, L.N. and ELVEHJEM, C.A. (1945): Intestinal synthesis of B vitamins by the rat. Arch. Biochem. 6: 403-410.
- SHARE, I.E., MARTYNIUK, E. and GROSSMAN, M.I. (1952): Effect of prolonged intragastric feeding on oral food intake in dogs. Amer. J. Physiol. 169: 229.
- SHARMA, K.N., ANAND, B.K., DUA, S. and SINGH, B. (1961): Role of the stomach on regulation of activities of the hypothalamic feeding centres. Amer. J. Physiol. 201: 593-598.
- SIMONNET, H., Le BAAS, H. and de BALSAC, H.H. (1953): cited by Mickelsen, O. Vitamins and Hormones. 14: 25; 1956.
- SMIDDY, F.G., GREGORY, S.D., SMITH, I.B. and GOLIGHER, J.L. (1960): Faecal loss of fluid, electrolytes and nitrogen in colitis before and after ileostomy. Lancet. 1: 14.
- SOUDERS, H.J. and MORGAN, A.F. (1957): Weight and composition of organs during the reproductive cycle in the rat. Amer. J. Physiol. 191: 1-7.
- SPERLING, L. (1938): Mechanics of simple intestinal obstruction. Arch. Surg. 36: 778-815.
- SPERLING, L. and WANGENSTEEN, O.H. (1935): Influence of obstruction of obstruction of the bowel upon its length and weight. Proc. Soc. Exper. Biol. & Med. 32: 1219-1226.
- STAHLGREN, L.H., UMANA, G., ROY, R. and DONNELLY, J. (1962): A study of intestinal absorption in dogs following massive bowel resection and insertion of an antiperistaltic segment. Ann. Surg. 156: 483-491.

- STANLEY, M.M. andd CHENG, S.H. (1956): Cholesterol exchange in the gastro-intestinal tract in normal and abnormal subjects. Gastroenterology. 30: 62-74.
- STANLEY, M.M. and CHENG, S.H. (1957): Excretion from the gut and gastro-intestinal exchange. Amer. J. Dig. Dis. 2: 628-642.
- STANLEY, M.M., COALSON, R.E., GROSSMAN, M.I. and JOHNSON, L.R. (1972): Influence of Secretin and Pentagstrin on acid secretion and parietal cell number in the rat. Gastroenterology. 63: 264-269.
- STRAUS, E., GERSON, C.D. and YALOW, R.S. (1974): Hypersecretion of gastrin associated with the short-bowel-syndrome. Gastroenterology. 66: 175-180.
- STROMINGER, J.L. (1947): The relation between water intake and food intake in normal rats and in rats with hypothalamic hyperphagia. Yale J. Biol. & Med. 19: 272.
- STUNKARD, A.J. and WOLFF, H.G. (1954): Correlation of arteriovenous glucose difference, gastric hunger contractions and the experience of hunger in man. Fed. Proc. 13: 146.
- SULLIVAN, M.F. (1965): Bile acid absorption in the irradiated rat. Amer. J. Physiol. 209: 158-164.
- SHARPE, S.J. and ROBINSON, M.F. (1970): Intermittent and continuous faecal markers in short-term metabolic balance studies in young women. Brit. J. Nutr. 24: 489-500.
- SYMONS, L.E.A. (1965): Kinetics of the epithelial cells and morphology of villi and crypts in the rat jejunum. Gastroenterology. 49: 158-168.
- TABAQCHALI, S. and BOOTH, C.C. (1966): Jejunal bacteriology and bile acid metabolism in patients with malabsorption either due to partial gastrectomy or to stagnant-loop syndrome. Lancet. 2: 12-15.
- TABAQCHALI, S., HATZIOANNOU, J. and BOOTH, C.C. (1968): Bile acid deconjugation and steatorrhoea in patients with stagnant-loop syndrome. Lancet. 2: 12-16.
- TAYLOR, A., PENNINGTON, D. and THACKER, J. (1942): cited by Mickelsen, O. in Vitamins & Hormones. 14: 22; 1956.
- TEEM, M.V., and PHILLIPS, S.F. (1972): Bile acids and absorption of water in the jejunum of the hamster. Gastroenterology. 62: 261-267.
- TEMPERLEY, J.M., STAGG, B.H., WYLLIE, J.H. (1971): Disappearance of gastrin and pentagastrin in the portal circulation. Gut. 12: 372-376.
- TEICHER, I. and ABRAHAMS, J.I. (1956): The treatment of selected cases of multiple polyps, familial polyposis, and diverticular disease of the colon by subtotal colectomy and ileoproctostomy. Surg. Gynae. & Obst. 103: 137-146.

- THOMAS, J.E., CRIDER, J.O. and MORGAN, C.J. (1934): A study of reflexes involving the pyloric sphincter and antrum, and their role in gastric evacuation. Amer. J. Physiol. 108: 683-700.
- TILSON, M.D. and WRIGHT, H.K. (1970): Adaptation of functioning and bypassed segments of ileum during compensatory hypertrophy of the gut in the rat. Surgery. 67: 687-693.
- TILSON, M.D. and WRIGHT, H.K. (1971): Humoral influence on villus hypertrophy. Clin. Res. 19: 405.
- TILSON, M.D. (1972): Compensatory hypertrophy of the gut: Testing the Tissue Mass, Intraluminal Nutrition, and Functional Demand hypotheses. Arch. Surg. 104(1): 69-72.
- TRENDELENBURG, P. (1917): Quoted by Sperling, L. in Arch Surg. 36: 778; 1938.
- TURNBERG, L.A. (1970): Electrolytes absorption from the colon. Gut. 11: 1049-1054.
- Van ITALLIE, T.B., BEAUDOIN, R.A. and MAYER, J. (1953): Metabolic hyperglycaemia and food intake in man: arteriovenous glucose difference. J. Clin. Nutr. 1: 208-216.
- WANGENSTEEN, O.H. and MINN, M. (1943): Primary resection of the colon and rectosigmoid. Surgery. 14: 403-432.
- WARREN, R. (1939): Serosal and mucosal dimensions at different levels of the dog's small intestine. Anat. Rec. 75: 427-437.
- WEBSTER, C.U. and HOWARD, R.R.S. (1973): Subtotal colectomy and caecoproctostomy. Brit. J. Surg. 60: 42-44.
- WEINER, I.M. and JACK, L. (1968): in Code, C.F. editor, Alimentary canal, vol. 3; Handbook of Physiology: Baltimore, 1968, the Williams & Wilkins Co.
- WEIRDA, J.L. (1942): Measurements and observations upon the intestine of rats fed unbalanced and supplemented diets. Amer. J. Anat. 70: 433-452.
- WEIRDA, J.L. (1950): A comparison of the weight of the intestine with the body and kidney weights in rats which were fed artificial unbalanced diets. Anat. Rec. 107: 221-231.
- WELCH, C.S., WAKEFIELD, E.G. and ADAMS, M. (1936): Function of the large intestine of man in absorption and excretion. Arch. Intern. Med. 58: 1110.
- WESSER, E. and HERNANDEZ, M.H. (1971): Small bowel adaptation after intestinal resection in the rat. Gastroenterology. 60: 69-75.
- WHITBY, L.G. and LANG, D. (1960): Experience with the chromic oxide method of faecal marking in metabolic balance investigations on humans. J. Clin. Invest. 39: 854.

- WHITSON, D., CARRICK, C.W., ROBERTS, R.E. and HAUGE, S.M. (1943): Utilization of fat by chickens - A method for determining the absorption of nutrients. Poultry Sci. 22: 137-141.
- WILMORE, D.W., DUDRICK, S.J., DALY, J.M. and VARS, H.M. (1971): The role of nutrition in the adaptation of the small intestine after massive resection. Surg. Gynae. & Obst. 132: 673-680.
- WINBON, W.B., SEELING, L.L., NAKAYAMA, H. and WESSER, E. (1974): Hyperplasia of the gastric glands after small bowel resection in the rat. Gastroenterology. 66: 384.
- WISEMAN, G., NEAME, K.D. and GHADIALLY, F.N. (1959): The effect of Sarcoma  $RD_3$  on intestinal active absorption of glucose and L-histidine. Brit. J. Cancer. 13: 282.
- WOOD, H.O. (1944): The surface area of intestinal mucosa in the rat and the cat. J. Anat. 78: 103-105.
- WRIGHT, H.K., CLEVELAND, J.C. and TILSON, M.D. (1969a): Morphology and absorptive capacity of the ileum after ileostomy in man. Amer. J. Surg. 117: 242-244.
- WRIGHT, H.K., POSKITT, T., CLEVELAND, J.C. and HERSKOVIC, T. (1969b): The effect of total colectomy on morphology and absorptive capacity of ileum in the rat. J. Surg. Res. 9: 301-304.
- YANG, M.G., MANOHARAN, K. and YOUNG, A.K. (1969): Influence and degradation of dietary cellulose in the caecum of rats. J. Nutr. 97: 260-264.
- YANG, M.G., MANOHARAN, K. and MICKELSEN, O. (1970): Nutritional contribution of volatile fatty acids from the caecum of rats. J. Nutr. 100: 545-550.
-