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MULTIPLE POLYPS AND COLONIC NEOPLASIA

AN EXPERIMENTAL ANIMAL MODEL

BY

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Thesis submitted for the degree of Doctor of Philosophy at the University of Glasgow, from the University Department of Surgery, Royal Infirmary, Glasgow.

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DEDICATION

To my wife Annette and son Peter Robert.

DECLARATION

I, Robert Carachi, declare that the thesis has been composed entirely by myself and that the work presented has been performed either on my own or in association with other research assistance as detailed below.

I also declare that I have personally checked the references entered in the text.

The experimental animal model (I) for colonic cancer which is recorded in CHAPTER TWO was carried out with the help of Dr A. Busuttil who assisted me in the histological assessment of the tumour induced. This work was presented at the Association of Clinical Pathologists, Stirling Royal Infirmary, Stirling, on 25th November 1976. A demonstration and a paper entitled "Experimental Colorectal Cancer" by R. Carachi, A. Busuttil and S.N. Joffe was presented as a preliminary communication.

The full paper was presented at the Pathology Society of Great Britain and Ireland. St George's Hospital, London, 6th January 1977. "An Experimental Model for colonic carcinoma". R. Carachi, A. Busuttil and S.N. Joffe.

Aspects of the development of the experimental model for colonic dysplasia (CHAPTER THREE), the toxicity of the carcinogen (CHAPTER FOUR) and the experiment showing the effect of dietary fibre (CHAPTERS FIVE AND SIX) was presented at the following learned societies:

British Society of Gastroenterology, Cambridge, 1st April 1977. "The Influence of a high and low fibre diet on the induction of colorectal tumours in the rat, using Dimethylhydrazine (DMH)." R. Carachi, A. Busuttil and S.N. Joffe.

Twelfth Congress of the European Society for Surgical Research, Warsaw, Poland, 25th April 1977. "The Influence of a high and low fibre diet on the induction of colorectal tumours in the rat using Dimethylhydrazine (DMH)." R. Carachi, A. Busuttil and S.N. Joffe.

Scottish Society of Experimental Medicine, Aberdeen, 3rd June 1977. "Dietary fibre and colorectal cancer". R. Carachi, A. Busuttil and S.N. Joffe.

Surgical Research Society, Manchester, 8th July 1977. "The protective role of dietary fibre in an experimental model for colorectal cancer". R. Carachi, A. Busuttil, S.N. Joffe and L.H. Blumgart.

The abstracts of these proceedings are published in the following journals:

The influence of a high and a low fibre diet on the induction of colorectal cancer in the rat using Dimethylhydrazine (DMH). R. Carachi, A. Busuttil, S.N. Joffe. The Journal of European Surgical Research, Vol. 9, p.193, 1977.

The influence of a high and a low fibre diet on the induction of colorectal cancer in the rat using Dimethylhydrazine (DMH).

R. Carachi, A. Busuttil, S.N. Joffe. GUT, Vol. 18, A410, 1977.

Dietary fibre and colorectal cancer. R. Carachi, A. Busuttil, S.N. Joffe. Scottish Medical Journal, Vol. 22, 3: 243, 1977.

The protective role of dietary fibre in an experimental model for colorectal cancer. R. Carachi, A. Busuttil, S.N. Joffe, L.H. Blumgart. British Journal of Surgery. Vol. 64, 837, 1977.

The definitive paper on the experimental animal model for colonic dysplasia (CHAPTER THREE) is published:

Multiple polyps and colonic neoplasia induced in the rat. R. Carachi, A. Busuttil, S.N. Joffe and L.H. Blumgart. Journal of Surgical Research, Vol. 29, No. 4, pp.363-370, 1980.

The tumour model is also registered and recorded in:

European Organization for the research into the treatment of cancer (E.O.R.T.C.). A registry of experimental digestive tumours in Europe. (1979). Page 21, No. 47.

CHAPTER ELEVEN. POLYAMINES AS TUMOUR MARKERS IN THE MODEL (II) is published:

Polyamines in colorectal cancer - a clinical and experimental approach. R. Carachi and J.G. Beeley. J. Clin. Pathol., 36, pp.508-510, 1983.

I submitted a review of my thesis for the British Association of Paediatric Surgeons inaugural prize for experimental work and was awarded it in 1981 (£600),

The paper was presented at the 29th Annual Congress of the British Association of Paediatric Surgeons, Madrid, Spain, 21st to 23rd July 1982 and the full text has been published as an article entitled;

Polyposis Coli: An Experimental Animal Model. Journal of Paediatric Surgery, Vol. 18, No. 1 (Feb), pp.51-57, 1983.

SUMMARY

Colorectal cancer is one of the commonest forms of malignant disease in man. The crude death rate in Scotland is 71 per 100,000 population and there has been no change in the incidence or the mortality of the disease over the past 20 years (Annual Report Registrar General for Scotland). There is a marked variation in the geographic incidence of colorectal cancer, Scotland having a sevenfold increased incidence than other communities (World Health Statistics Annual). The younger patients who develop the disease may have underlying conditions such as polyposis coli and adenomas of the colon which predispose them to develop colonic dysplasia and neoplasia, in contrast to the majority of those in their late middle age who have no apparent predisposing factors.

It is with the former younger group of patients in mind that this thesis is submitted, using an appropriate animal model for colonic dysplasia, in an attempt to answer questions on the aetiology of these tumours. Although animal models have been used to study cancer, they were unsatisfactory models for studying colonic dysplasia and neoplasia. The aim of this thesis is to establish a model with an unstable colonic epithelium for colorectal dysplasia to develop, in order to study the effects of the colonic intraluminal environment on this dysplasia.

The experimental model for colorectal cancer described (Martin et al. 1973) was used as a basis for the model and their experiment was repeated (CHAPTER TWO). Colorectal neoplasia were induced by using

the carcinogen 1:2 Dimethylhydrazine (DMH) injected at a dose of 15 milligrams per kilogram body weight weekly for 28 weeks. This model was modified by reducing the total carcinogen dose to weekly injections for only 16 weeks which produced colonic dysplasia, less extra colonic tumours and minimal carcinogen toxicity. Focal areas of dysplasia underwent changes to polypoidal cancers after stopping the carcinogen at 16 weeks (CHAPTERS THREE AND FOUR). This formed the basis of the animal model modified for epithelial dysplasia (II) used in the rest of the thesis.

The experimental model was used to test the hypothesis that by alteration of the colonic intraluminal environment, in this model for colonic dysplasia, the natural history of the disease could be modified (CHAPTER FIVE). The effect of a high fibre diet on the experimental model proved that fewer tumours appeared and they developed later than rats on a normal diet. Tumours induced in rats on a high fibre diet were morphologically less aggressive and better differentiated histologically. Dietary fibre appears to be one factor which protects the colonic mucosa in the model from the carcinogen 1:2 Dimethylhydrazine (DMH) (CHAPTER SIX).

The next set of experiments carried out on the model was to measure the faecal bile acids which have been reported as co-carcinogenic (Hill 1974). The total faecal bile acid excretion was significantly lower in rats on a high fibre diet receiving carcinogen than those similarly injected but on a normal diet or in control animals. These measurements were carried out at six intervals during the 28 weeks of the experiment (CHAPTER SEVEN). The intestinal transit time was

measured in a similar experiment on the model, since intestinal transit time has been reported as an explanation for the regional variation in the incidence of colorectal neoplasia (Burkitt 1971). The experiment showed a significant decrease in the intestinal transit time in the rats on a high fibre diet (CHAPTER EIGHT). The faecal weight and bulk was measured in a similar experiment with rats on a high and low fibre diet. The mean faecal residue in rats on a high fibre diet was significantly greater in weight and volume than those on normal diets whether they received carcinogen or not (CHAPTER NINE). The post mortem findings in this last group of animals showed that rats fed on a high fibre diet were found to have fewer tumours ($p<0.01$) and less mass of tumour was induced (1.4 grams as opposed to 9.6 grams) than rats fed on normal diet receiving carcinogen (CHAPTER TEN). Finally, as a clinical application, the experimental model for colonic epithelial dysplasia was used to test the value of Polyamines as tumour markers after measuring them in patients with colorectal cancer (CHAPTER ELEVEN).

The experimental animal model developed for the purpose of studying colonic neoplasia developing from colonic dysplasia has been proved to be reliable and was used to test the hypothesis that alterations in the colonic intraluminal environment can modify the natural course of the disease in this model. It is hoped that this experimental evidence may be of clinical significance to young patients at risk of developing neoplasia because of their predisposition to colonic dysplasia.

Robert Carachi

GENERAL INTRODUCTION

I. Historical Review

It is extremely important to ask whether a tumour model for multiple polyps and colonic neoplasia is relevant to the study of the human disease. Significant contributions have been made to the problem of the aetiology of colorectal cancer, as well as an understanding of some of the causative factors in neoplastic disease by studies of the geographic pathology of these tumours (Segi et al 1969). Cancer of the colon and rectum exhibit sizable differences in incidence and in death rate as a function of the geographic area (Weisburger 1971). The epidemiological evidence points to the fact that colorectal cancer is heavily influenced by environmental factors. This variation ranges from areas with a high incidence, as in Scotland and the United States, to those areas of very low incidence as in rural Africa where it is virtually a rare disease (Illustration 1).

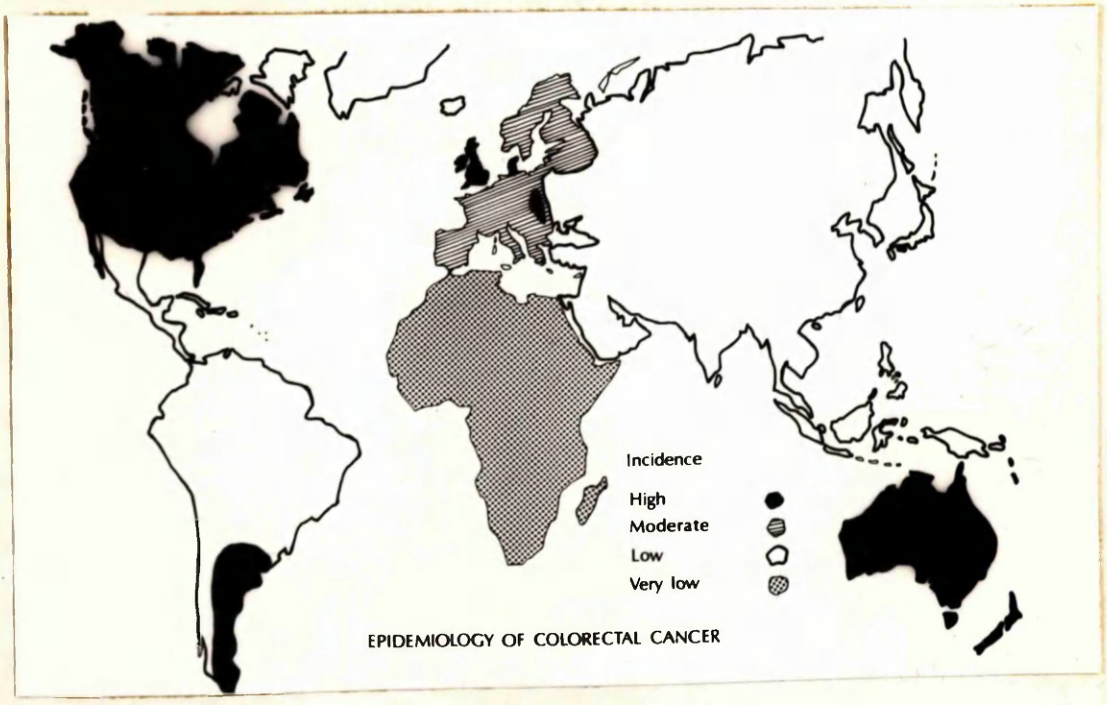


Illustration 1

Migration studies have shown that the immigrant population take on the risk factor of the area they inhabit and that it is not an inherent factor in the people themselves. This epidemiological evidence has favoured environmental carcinogens and environmental factors (promotors) as aetiological agents in the natural history of colorectal cancer. (Haenszel and Correa 1971)

In a number of instances, cancer at various organ sites in man has been connected to the occupational exposure to specific chemicals, e.g. bladder cancer in dyestuffs and rubber workers, and lung cancer in uranium workers, etc. This has not been the case with colorectal neoplasms. In colon cancer, prime consideration centres around dietary and nutritional elements which are different in regions of high and low incidence of the disease. Lack of dietary fibre has been implicated in the aetiology of colorectal disease in general and colonic neoplasia in particular (Burkitt 1971). The high association of meat consumption with the incidence of colorectal neoplasms has also been reported (Haenszel et al 1974). The reports concerning the high mortality rates for colonic cancer among the Japanese migrants to the United States have lent strength to the fact that colonic and rectal tumours are an environmentally induced disease (Haenszel et al 1973). Endogenous carcinogenic factors may also play a significant role in the aetiology and pathogenesis. High faecal bile acid concentrations in the presence of faecal Clostridia capable of dehydrogenating the bile acid nucleus was found in patients with colorectal neoplasia. These factors could present to the mucosa of the large bowel, carcinogenic moieties whose actions are enhanced by the presence of high sterol concentrations or other environmental

cofactors essential to the process of carcinogenesis (Hill 1974). Although a beginning has been made in the studying of carcinogenesis in man, detailed information so far has come chiefly from studies using laboratory rodents. Ideally the type of colorectal neoplasm induced in animals should have close anatomical, pathological and morphological similarity to those in man. They should be associated with polyps, the times of induction should be short, activation should be similar to that in man, multiple species should be susceptible, the effective carcinogens should be relatively specific for the colorectum and should be related to the agents in the environment that may be the factor for the clinical disease (Burdette 1974).

The incidence of spontaneous colonic cancer in rats has been reported at 0.1% (Miwa et al 1976). Among experimental animals only the hamsters, in addition to the rats, have been reported to have other than rare spontaneous polyps and adenocarcinomas of the colon (Fortner 1968). Otherwise, tumours used extensively in the laboratory have been chemically induced. An example long known to cause cancer in the colon, rectum and other tissues in animals, is bracken fern (Pamukcu et al 1970). A micro-organism demonstrated to be carcinogenic is Aspergillus flavus, since aflatoxin B1 is known to produce neoplasms of the colon and rectum in rats in the presence of vitamin A deficiency (Newbern and Rogers 1973). In early studies, aromatic carcinogenic hydrocarbons were not very effective intestinal carcinogens unless special techniques for localising them in the colon such as anchoring impregnated sutures in the gut were used (Burdette 1965). It is now known that different strains of mice, rats and hamsters have different susceptibilities to neoplasms induced with

these compounds. Results may differ between the sexes in the same strain (Homburger et al 1971).

Investigations in the structure-activity relationship with a number of aromatic amines revealed that the methyl substituted 4-aminobiphenyls yield cancer of the colon in rats in fair yields with a latent period of one year (Walpole and Williams 1958, Walpole 1960). The carcinogen utilised most frequently in this group has been 2'3-dimethyl-4-aminobiphenyl.

Cycasin - The carcinogenicity to the colon as well as liver and kidney of a natural product, cycasin, the b-glucoside of methylazoxymethanol was discovered by serendipity (Laqueur 1965). The cycad meal is sometimes used after ineffective detoxification by native groups in Guam and Africa. It was suspected to be the cause of amyotropic lateral sclerosis in man and when given chronically to rats in their diet, failed to induce this condition but instead, led to cancer, especially in the lower intestinal tract. Laqueur demonstrated that the bacterial flora in the lower gut is uniquely involved in the release of the carcinogenic intermediate methylazoxymethanol because cycasin itself is ineffective in germ free animals. On the other hand, injection or oral administration of the aglycone methylazoxymethanol, even in germ free rats, yielded the same type of cancer as when cycasin was administered to conventional rats although not in the same yield. (Laqueur 1970)

Druckrey and associates (1967, 1970, 1972) developed another colon specific carcinogen on the basis of the reports by Laqueur and his

associates about cycasin and so the powerful carcinogenicity to the bowel of 1,2-dimethylhydrazine was discovered. This agent as well as the closely related azoxymethane induced a high incidence of the cancer in the colon after a latent period of 200 to 300 days, depending on the dosage. It was discovered that if the compound was given orally, it also leads to cancer of the liver and kidneys, whereas given subcutaneously, it yielded mainly cancers of the colon, some in the rectum and the small intestine, especially the duodenum. Mice and hamsters were also found to be susceptible.

Previous attempts at inducing colorectal cancer in a laboratory animal were unsuccessful due to lack of specificity and organotropism of the carcinogen for colonic mucosa. The discovery by Laqueur of the carcinogenic effect of cycasin and its aglycone on intestinal mucosa opened the way to the development of new synthetic carcinogens: N'-methyl-N-nitro-N-nitrosoguanidine (Narisawa et al 1971), N-methyl-N-nitrosourea (Narisawa et al 1975), methylazoxymethanol acetate (Reuber 1976) in attempts to produce a more specific model with less toxicity.

Tumours have been induced in inbred and random bred rats (Spjut and Noall 1971), mice, guinea pigs (Schauer et al 1969) and hamsters (Ward et al 1973). The other routes of administration have been by intra-rectal administration (Narisawa et al 1975), as a single-loading dose (Reuber 1976) or separate doses given over a period of time (Spjut et al 1971). The important factors to consider in tumour induction in an experimental animal include the type of carcinogen, the mode of administration of the carcinogen and the species and sex of the

animals used. Most of the carcinogens listed except for dimethylhydrazine and its metabolites, azomethane and methylazoxymethanol, produce a low incidence of intestinal adenocarcinomas and the induction of extraintestinal tumours is common (Narisawa et al 1971). The mode of administration is important since a single injection of methylazoxymethanol produces colonic polyps as well as small bowel tumours (Reuber et al 1976). An intrarectal infusion of the same carcinogen produced squamous cell carcinomas and myosarcomas (Schauer et al 1969). Administration of dimethylhydrazine in a dose of 15 milligrams per kilogram body weight for a period of seven months produced both small and large bowel tumours in addition to hepatotoxicity in 60% of the rats with development of fibrosarcomas (Narisawa et al 1975). When a total lower dose of methylazoxymethanol was administered intraperitoneally, male rats developed multiple polypoidal carcinomas in the left colon (Weisburger 1971).

The model I decided to use in this thesis is the experimental model for cancer of the colon and rectum. Intestinal carcinoma induced in the rat by 1,2-dimethylhydrazine. This paper was reported in "DIGESTION" 8: 22-34 (1973) by M.S. Martin, F. Martin, R. Michiels, H. Bastien, E. Justrabo, M. Bordes and B. Viry from the Faculty of Medicine and Centre Regional de Lutte Contre le Cancer, Dijon. This model was chosen because of the reports of superiority of dimethylhydrazine in its specificity for the intestine, the very high incidence of colorectal cancer reported, the organospecificity of the compound and the relative short induction time of the tumours. This carcinogen was also commercially available and was reported by these authors to give no incidence of extra intestinal tumours and therefore

had definite advantages over other carcinogenic agents. In the paper they reported administering 1,2-dimethylhydrazine (DMH) through a subcutaneous route to a group of 20 inbred BD-IX rats at the dose of 15 mg/kg body weight, weekly for seven months. Intestinal adenocarcinomas were found in all the treated animals, often associated with hyperplastic or dysplastic lesions. The intestinal cancers were reported to give metastases in 14 of the 20 animals. The authors reported a low incidence of extra intestinal malignancies, a relatively short induction time, and a striking similarity to human colorectal adenocarcinomas, all of which made DMH induced intestinal cancer a highly effective experimental model.

The authors reported using 20 inbred rats from BD-IX strain (9 males and 11 females) in their experiment. The strain had been maintained by brother-sister inbreeding over 50 generations and histocompatability, checked by tolerance of skin transplant. The rats aged 9 weeks were allowed a week to get used to the surroundings in the animal room before beginning treatment. They were separated according to sex and housed in cages in groups of three or four. The animals were fed a standard pellet diet (biscuit Extralabo) and given tap water (ad libitum).

DMH hydrochloride was dissolved immediately prior to injection in demineralised water in the concentration of 16.6 mg/ml then neutralised at pH6 by sodium hydrogen carbonate after adding ethylene diaminotetracetate (0.5 mg/ml) for stabilization. The rats were given weekly subcutaneous injections of 15 mg/kg body weight. The doses were calculated to the DMH base (0.45 mg per 1 mg of DMH dihydrochloride).

The injections were discontinued after 28 weeks of treatment and a total dose of 420 mg/kg body weight was given. The rats were weighed weekly at the same time of the day and a daily watch was kept for the appearance of clinical symptoms of cancer. Two of the 20 animals were found dead in their cage. The 18 remaining rats were killed by ether before symptoms announcing spontaneous death. Gross and histological examination of the abdominal and thoracic viscera were carried out on each animal.

Clinical symptoms of intestinal lesions appeared in some rats as early as 18 weeks after initiation of treatment: retarded growth, weight loss, roughness of the fur, diarrhoea, bloody stools, subocclusion; ear lesions led to loss of balance in two rats.

Length of survival after initiation of treatment varied from 203 to 323 days and was longer in females (mean 282 days) than in males (mean 235 days). This difference was reported as highly significant by the authors ($p<0.001$).

The intestinal lesions that were induced were classified into three groups: (TABLE I)

1. Intestinal cancers were always carcinomas except in one rat which showed three leiomyosarcomas of the colon and rectum associated with four carcinomas. The carcinomas were usually multiple and increased in animals with the longest survival. Thirteen of the 20 rats developed one or several carcinomas of the small intestine, very often localised to the duodenum, a few centimeters from the

pylorus. Carcinomas of the colon were observed in all the rats in this series; localisation to the rectum was relatively rare. No significant difference was found between males and females in the location or number of the carcinomas. The gross appearance of the lesions varies from sessile tumours infiltrating the wall of the colon, polypoidal carcinomas able to obstruct the intestinal lumen, occasionally associated with intussusception. The size of the tumours reached 13 mm. Histologically these cancers were usually adenocarcinomas, often well differentiated: mucoid carcinoma, signet ring cell carcinoma were also observed. Invasiveness of submucosa through muscularis mucosa was the minimum criterion used by the authors to classify a lesion as carcinoma. They reported that many of the lesions involved all the layers of intestinal wall including the serosa. In 14 of the 20 rats, metastases occurred in abdominal lymph nodes, lung, omentum or diaphragm.

2. Benign hyperplastic lesions were rarer than carcinomas and there were two hyperplastic polyps.

3. Dysplastic lesions: the authors included in this group lesions limited to the mucosa but differing from hyperplastic lesions by atypical features: cellular basophilia, crowded and hyperchromatic nuclei, greater density of the glands. These lesions were plaque-like or polypoidal. They reported it difficult to draw an exact line in this group between carcinoma in situ and precancerous or benign lesions. The distribution of these dysplastic lesions was roughly parallel to that of carcinoma.

Extra-intestinal lesions occurred in DMH-treated rats and a few of them were cancerous:

Stomach: Benign hyperplastic lesions occurred in the rumen (4 rats) or the glandular stomach (7 rats).

Liver: It was abnormal in 17 of 20 animals. Degenerative change of hepatocytes, sometimes with necrosis and haemorrhage occurred in 13 rats. A cystic distension of bile ducts (benign cholangioma) was observed in 7 animals.

Liver fibrosarcoma with ascites and metastasis to omentum occurred in one rat.

Ear duct: Five rats (one male and four females) developed tumour of the ear duct: these tumours were benign in four cases (3 sebaceous adenoma, 1 papillokeratoma), malignant in one case (squamous cell carcinoma).

Other organs: Gross and histological examinations of other organs (kidney, pancreas, spleen, lung, heart, bone marrow, thymus, supra-renal glands, central nervous system) showed no tumour. No cancer was observed in the skin at the site of injection of the DMH.

	Carcinomas	Dysplastic lesions	Hyperplastic lesions
Caecum	13/20 (13+)	3/20 (4)	3/20 (4)
Transverse	16/20 (31+)	3/20 (3)	1/20 (1)
Descending	14/20 (24)	4/20 (5)	1/20 (1)
Rectum	5/20 (5+)	2/20 (3)	1/20 (1)
Total	20/20 (73)	9/20 (15)	5/20 (7)

TABLE I

COLONIC TUMOURS INDUCED BY DMH (MARTIN ET AL 1973).

This table gives the proportion of affected animals and the number of observed lesions (between brackets) for each localization and each type of lesion (+ = one leiomyosarcoma).

In view of the epithelial dysplasia and the high incidence of polypoidal adenocarcinomas induced in their experimental model, I repeated their experiment (Chapter 2 THE EXPERIMENTAL ANIMAL MODEL) in an attempt to reproduce their experimental results. This basic experimental animal model was then modified to induce colonic dysplasia alone (CHAPTER THREE).

The carcinogens used in the experimental models may act either directly on the epithelium or require metabolic activation. Adenocarcinomas of the rectum and colon were produced by local application of N-methyl-N'-nitro-N-Nitrosoguanidine (MNNG) by rectal irrigation in Donryu rats. Both polyps and adenocarcinomas were found in the region that came in direct contact with the carcinogen (Narisawa 1972). Numerous studies on the binding of carcinogenic hydrocarbons have been carried out since early studies revealed protein bound amino azo dyes in the livers of rats fed on the hepatocarcinogen N,N-dimethyl-4-aminoazobenzene, with some correlations between the amounts present and the likelihood of the appearance of neoplasms (Miller and Miller 1966). Subsequent studies have shown that various carcinogens bind covalently with DNA, RNA and protein but the degree of binding with a specific target tissue does not necessarily correlate well with the carcinogenic properties of the compound for the target (Miller and Miller 1972). Thus, the relationships between the ultimate steps of carcinogenesis and mutagenesis are not clearly established.

Parallels exist between the human and DMH-induced carcinogenesis of colorectal neoplasms and it is important to establish them in order that the various experiments carried out in this thesis will be

relevant to the clinical problem.

Parallel One: The carcinogen used, 1,2-dimethylhydrazine, undergoes conjugation of the metabolite with glucuronic acid, secretion of the conjugate in bile and liberation of the free N-hydroxy compound by action of the bacterial flora in the large intestine, where reaction must take place with the colonic mucosa (Weisburger 1971). Diversion of the faecal stream will prevent the development of the neoplasm in rats undergoing experimental carcinogenesis (Navarette and Spjut 1967). Thus, a parallel exists between the epidemiological presumptions concerning the development of colorectal neoplasms, the presence of endogenous carcinogens, bacterial metabolism capable of inducing colorectal cancer in humans and the experimental evidence for these requirements in DMH carcinogenesis in the rat model.

Parallel Two: The cell kinetics of neoplastic lesions both in humans and in this experimental model have shown that the process of carcinogenesis using dimethylhydrazine is associated with auto-radiographic changes reflecting a decrease in the number of mucus secreting cells. There is also inhibition of differentiation of basal epithelial cells with an increase in the number of DNA synthesising cells in the proliferative zone of the crypts of Lieberkuhns (Springer et al 1970). These findings extended the whole length of the villi, showing high proliferative activity. Papillomas developed in those areas closely related to the proliferative activity of the epithelium. Others have shown similar changes resulting in net cell "retention" in the mucosa of human colonic neoplasms (Deschner and Lipkin 1971).

Parallel Three: The histopathology of the carcinogen induced carcinoma of the colon is remarkably similar to the human counterpart. Multiple synchronous tumours are induced which correspond to the development of colorectal cancer in multifocal sites in dysplasia of the colon as is seen in polyposis coli (Ward et al 1973).

Parallel Four: Immunological evidence shows the presence of detectable circulating antigens in carcinoma of the colon, both in humans and in the experimental models. Species specific carcinoembryonic antigen has been searched for in DMH-induced colorectal carcinoma in the rat (Garmaise et al 1974 and Thomson et al 1969). Thus, evidence for the presence of oncofetal antigens detectable by immunodiffusion, radio-immunoassay or the ability to act as blocking factors in micro-cytotoxicity assay have been demonstrated in both human and DMH-induced colon cancer (Steele and Sjogren 1974). These private or neoantigens are shared both by human tumours of the same histological type and by DMH-induced colorectal carcinomas of the same histological type (Enker and Jacobitz 1976).

From the evidence presented in this historical review it is clear that striking parallels do exist between the DMH-induced animal model of colon cancer in the rat and the human disease of colorectal neoplasia developing in a setting of colonic dysplasia as is seen in polyposis coli. Because of this unique similarity, it is reasonable to believe that experimental data in this thesis resulting from a modification of this model will prove relevant in the better understanding of the disease.

II. Experimental Colonic Carcinogenesis.

Reliable induction of colon cancer can be achieved with two broad classes of chemicals:

a. Aromatic amines, derivatives of 3-methyl-4-amino biphenyl.

b. Derivatives of 1,2 dimethylhydrazine which includes the plant cycasin.

A large number of bowel carcinogens are now known for laboratory animals. Experimental studies of cancer in these animals have revealed that with different regimes cancer of the bowel may be induced at one site only or at two or more sites and these may or may not be accompanied by tissues and organs outside the bowel developing cancers. Distribution patterns of the multiple cancers induced by specific regimes in laboratory animals are constant and reproducible (TABLE II). Benzidine fed to rats induces cancer of the rectum and rectosigmoid junction. In rats that have ingested cycasin from the cycad seed of the primitive palm tree, most of the cancers arise in the caecum and in the colon. Polyurethane injected intraperitoneally induces cancer of the caecum and the transverse colon. By changing various factors such as the carcinogen, the solvent, the route of administration, the species and the strain of laboratory animal, the microflora of the gut, diversion of the faecal stream, enzyme induction in the liver, it is possible to induce cancer in different segments of the alimentary tract to modify the site and to influence the incidence of tumours. It is possible, theoretically, that human beings could ingest carcinogens or inhale or absorb substances and excrete the metabolites, either through the biliary system or by way of the bowel mucosa, thus setting the stage for the induction of cancer of the colon.

Carcinogen	Animal	Route	Caecum	Ascending Colon	Transverse Colon	Sigmoid Colon	Rectum
D.M.A.B.	Rat	S.C.				*	
4-Aminobiphenyl	Rat	S.C.	*			*	
Fluorenelyne							
Bisacetamide	Rat	O.	*	*			
Cycasin	Rat	O.	*	*			
3-Methylcholanthrene	Hamster	O.	*			*	
Benzidine	Rat	O.					*
Yttrium	Rat	O.				*	
Polyurethane	Rat	I.P.	*		*		

TABLE II
EXPERIMENTAL TUMOURS OF THE COLON
INDUCED BY CHEMICAL AND PHYSICAL AGENTS (STEWART 1971).

(a) Aromatic amines, 2', 3-Dimethyl-4-aminobiphenyl - Investigations in the structure activity with a number of aromatic amines revealed that methyl substituted 4 aminobiphenyls yield cancer of the colon in rats in a good yield over a year. The compound most commonly used is 2',3-dimethyl-4-aminobiphenyl. Adenomatous polyps and carcinomas of the large intestine have been induced by the subcutaneous injection of 2',3-dimethyl-4-aminobiphenyl (DMAB) (Illustration 2).

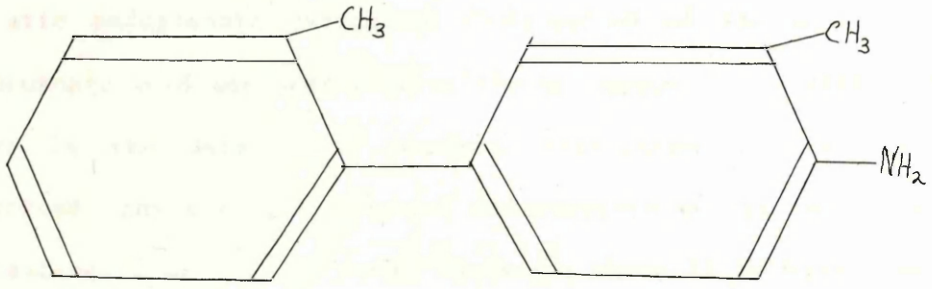


Illustration 2

2'3-Dimethyl-4-aminobiphenyl (DMAB)

The macroscopic and microscopic morphology of the tumours induced was noted to be similar to that in the human. The segmental distribution throughout the colon and rectum bore a similarity to that found in human colonic cancer. The transition from benign tumour to carcinoma was not demonstrated. Radioactive labels DMAB - SH has been used to study tissue distribution and retention of the carcinogens. It is

believed that the effectiveness of DMAB is largely due to a metabolite, probably metabolised in the liver. Workers are now in the process of trying to identify its trace metabolites (Spjut and Spratt 1965).

Metabolism of 4-Aminobiphenyls: The metabolism of 4-aminobiphenyl derivatives involves acetylation, N-hydroxylation at the 4' position and hydroxylation of the nitrogen, yielding N-hydroxy-4-acetylaminobiphenyl. It is this agent that is the active carcinogenic intermediate. The steps in the conversion of 4-aminobiphenyls into the active carcinogenic compound in the gut occurs in the liver by the hepatic endoplasmic reticulum. Conjugation of the metabolites with glucuronic acid and secretion of the conjugate in the bile is the next step in the degredation process. Liberation of the free active compound, the postulated active intermediate occurs by the action of intestinal flora in the large intestine where it is hydrolysed by the bacterial enzyne b-glucuronidase. The carcinogenic ion released reacts with the target tissues, cells and molecules of the cell to induce carcinogenic changes (Illustration 3).

Carcinogens may act directly on the epithelium or require metabolic activation. Narisawa produced adenocarcinoma of the rectum and colon by local application of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) by rectal irrigation. Both polyps and adenocarcinomata were found in the region in direct contact with the carcinogen. A number of studies on binding of carcinogenic hydrocarbons have been done since Miller et al showed that protein bound aminoazodyes in the livers of rats fed with hepatocarcinogen N,N-dimethyl-4-aminoazobenzene with correlations of the amounts present and the appearance of neoplasm.

Various carcinogens bind covalently with DNA, RNA and protein, but the degree of binding with a specific target tissue does not necessarily correlate with the carcinogenic properties of the compound for the target cell. Therefore the relationship between the ultimate steps of carcinogenesis and mutagenesis are far from clear at the target cellular level, even though the metabolic pathway for the particular carcinogen may have been worked out.

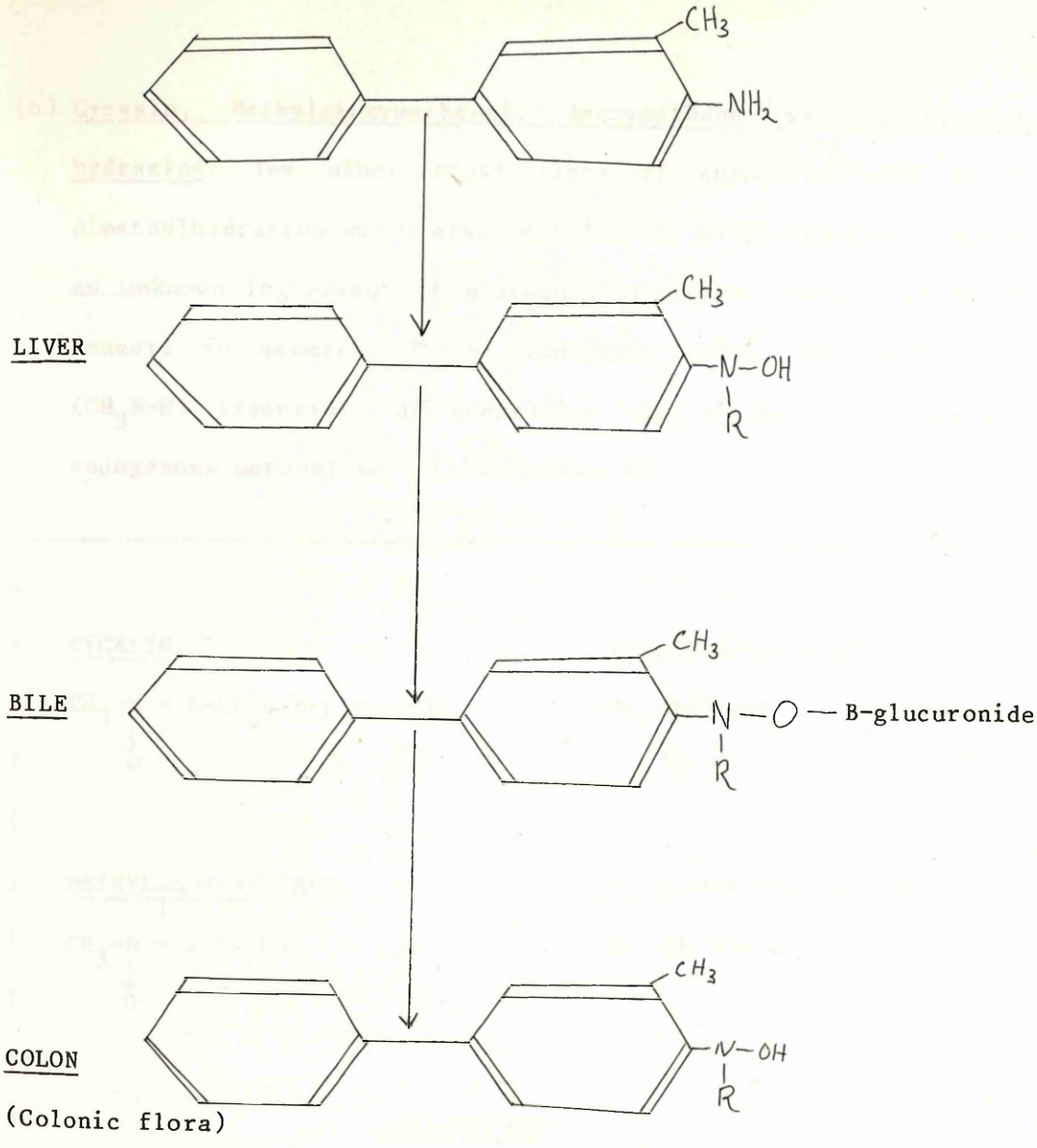


Illustration 3

Metabolism of 4-aminobiphenyls

(b) Cycasin, Methylazoxymethanol, Azoxymethane and 1,2-Dimethylhydrazine: The other broad class of chemicals used is 1,2 dimethylhydrazine which also includes the plant cycasin. Sometimes an unknown ingredient of bracken fern causes caecal and colonic cancers in animals. These components share the configuration (CH_3N-N) essential for generating the ultimate carcinogen by endogenous metabolism. (Illustration 4)

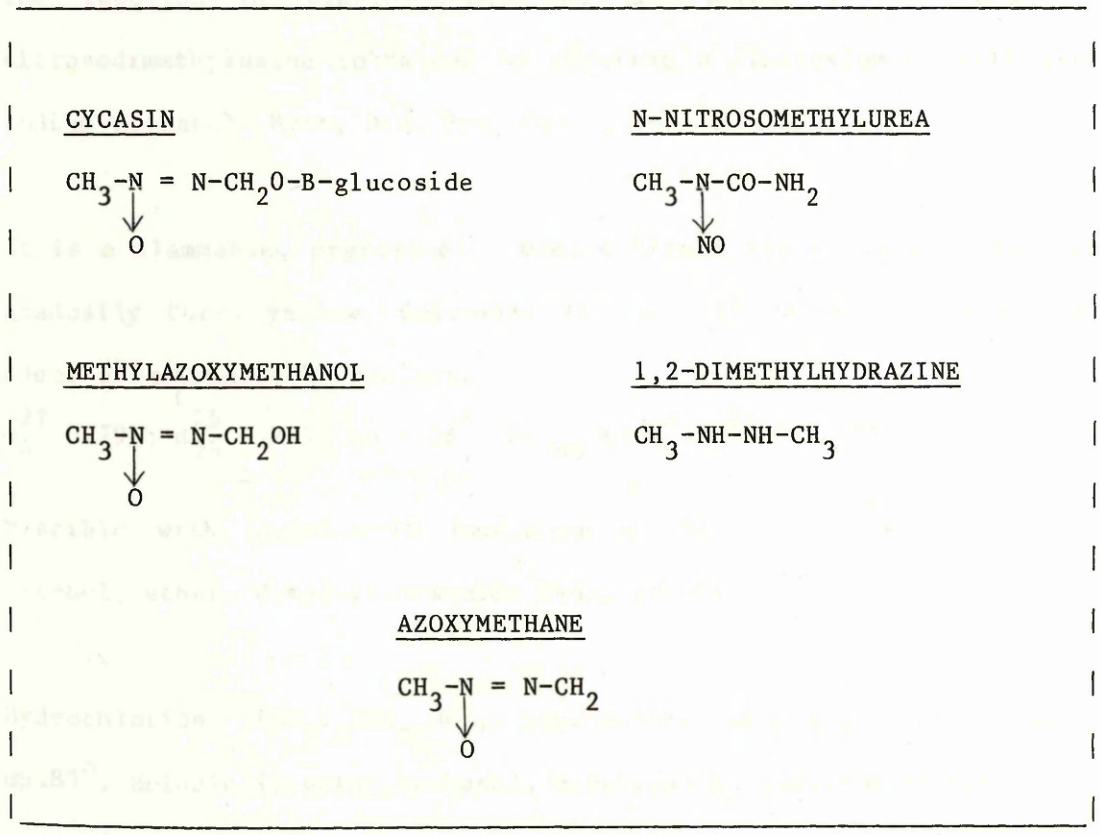


Illustration 4

Chemical Formulae of gastrointestinal carcinogens

The physical and chemical properties of the Dimethylhydrazine derivatives are outlined below:

1,1-Dimethylhydrazine: Unsym-Dimethylhydrazine: asymmetric dimethylhydrazine: N,N-dimethylhydrazine: Dimazine: UDMH $(CH_3)_2NNH_2$: mol wt 60.10. $C_2H_2N_2$: C39.97%, H 13.42%, N 46.62%. Prepared industrially from dimethylamine and ammonia in the presence of suitable catalysts: by the reaction of dimethylamine and chloramine: by reduction of nitrosodimethylamine (obtained by treating a dimethylamine salt with sodium nitrate): Hatt, Org. Syn. Coll., Vol. 2, 211 (1943).

It is a flammable, hygroscopic, mobile liquid which fumes in air and gradually turns yellow. Corrosive to skin: characteristic ammonical odour of aliphatic hydrazines.
d_4^{22} 0.791; d_{25}^{25} 0.782. mp - 58°. bp_{760} 63.9°. $n_D^{22.2}$ 1.40753.

Miscible with water with evolution of heat. Also miscible with alcohol, ether, dimethylformamide hydrocarbons.

Hydrochloride, $(CH_3)_2NNH_2$,HCL, hygroscopic crystals from ethanol, mp.83°. Soluble in water, ethanol, practically insoluble in ether.

Use: The base of rocket fuel formulations. Human toxicity. Highly corrosive and irritating to skin, eyes, mucous membranes.

1,2-Dimethylhydrazine: Sym-Dimethylhydrazine; N,N'-dimethylhydrazine. $CH_3NHNHCH_3$: mol wt 60.10. $C_2H_3N_2$: C39.97%. H13.42%, N46.62%. Preparation from dibenzoylhydrazine: Folpmers, Rev. Trav. China. 34,

34 (1915): Hatt, Org. Syn. Coll. Vol 2, 208, (1943).

It is a flammable, hygroscopic, mobile liquid which fumes in air and gradually turns yellow. Corrosive to skin: characteristic ammonical odour of aliphatic hydrazines.
d_4^{20} 0.8274, bp_{753} 81° n_D^{20} 1.4209.

Miscible with water with much evolution of heat. Also miscible with alcohol, ether dimethylformamide, hydrocarbons.

The 1,2-Dimethylhydrazine-Dihydrochloride compound, $C_2H_8N_2$.HCL is extremely hygroscopic, flammable, fumes in air and gradually turns yellow on standing. It is freely soluble in water, ethanol. It has corrosive and carcinogenic properties. It has no known economic value at present, unlike the 1,1-Dimethylhydrazine which is used as a base for rocket fuel formations and does not have the carcinogenic potency of this sister compound.

Cycasin: The carcinogenicity to the colon is well known. It is also toxic to the liver and kidney. It is the B-glucoside of methylazoxymethanol which was discovered by serendipity by Lacqueur. Cycasin was suspected to cause amyotropic lateral sclerosis in man and was given to rats in their diet which led to cancer of the gastrointestinal tract. Lacqueur showed that the bacterial flora in the colon is involved in the intermediate methylazoxymethanol because cycasin alone is inactive in germ free animals. Injection or oral administration of the aglycone methylazoxymethanol, even in germ free rats, yielded the same type of cancer as when cycasin was given to

ordinary rats.

1,2-Dimethylhydrazine and Azoxymethane: Druckrey developed another colon specific carcinogen on the basis of Laqueur's work with cycasin. This agent closely related to azoxymethane induced a high incidence of cancer of the colon after a latent period of 200 to 300 days, depending upon the dosage used. Orally, it produced cancers of the liver and kidney and given subcutaneously, it yielded mainly cancers of the colon and rectum, depending upon the dose, as well as small intestinal tumours.

Metabolism of Dimethylhydrazine (DMH)

The second broad group of carcinogenic compounds yield azoxymethane. Thus, after a subcutaneous injection, 1,2-Dimethylhydrazine enters the blood stream and is then metabolised by the endoplasmic reticulum in the hepatocytes. Hydroxylation in the liver changes the compound into the bioactive amines, azomethane and azoxymethane. These compounds are then conjugated and excreted as B-glucuronide in the bile. The enzyme B-glucuronidase in the colonic bacterial flora breaks down the metabolite and converts this compound to methylazoxymethanol. In germ-free animals this does not occur and most of the dimethyl-hydrazine can be collected in the urine and faeces and no tumours develop. Spontaneous breakdown to methyldiazonium then occurs and the methonium ion is believed to be the final alkylating agent for carcinogenesis. (Illustration 5)

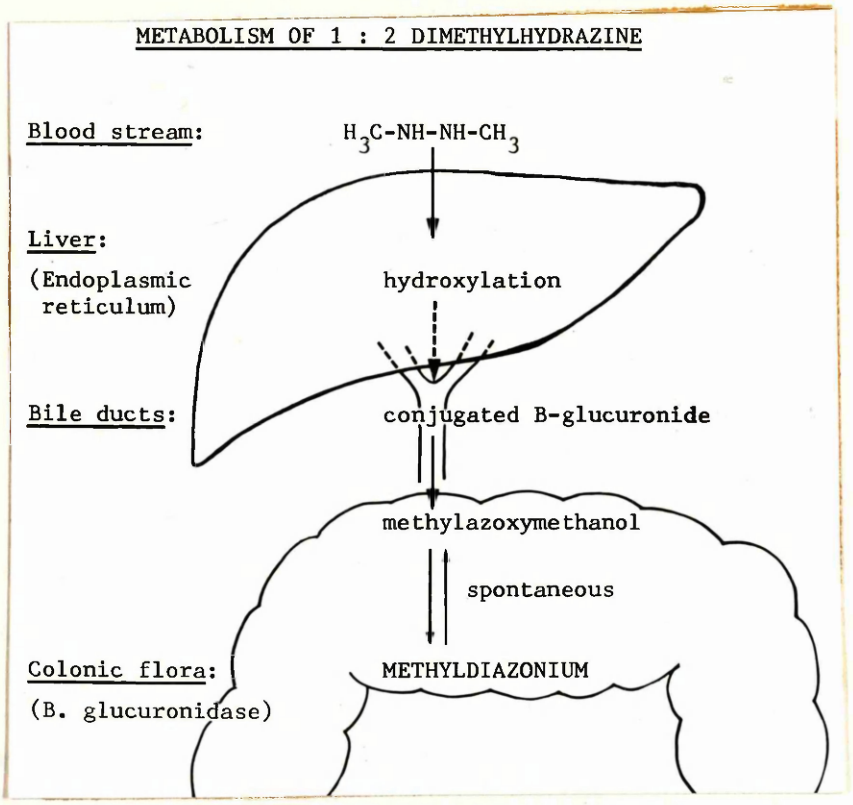


Illustration 5

Metabolic pathway of 1:2 Dimethylhydrazine (DMH)

Additional steps in the production of methyldiazonium and the methonium ion suspected to be the ultimate carcinogenic compound and active ion respectively are illustrated below (Illustration 6).

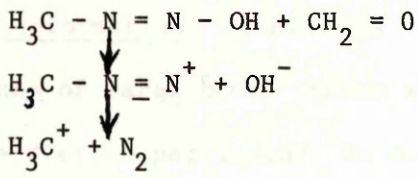




Illustration 6

Bioactive radical for carcinogenesis

III Theory and Hypothesis

I propose to construct a hypothesis of the possible development of events that patients with colonic epithelial dysplasia experience, which is consistent with the clinical, pathological, epidemiological and experimental information available.

Clinical information: Patients with heredofamilial polyposis coli, long standing ulcerative colitis, colon cancer families, adenomas of the colon and patients with ureterosigmoidostomies for urinary diversion, develop colonic epithelial dysplasia because of a predisposition genetically or otherwise to an unstable colonic epithelium. These patients are a high risk group for developing colorectal cancer at a young age which has been shown to develop from

these areas of dysplasia. It is usually a multifocal cancer and is associated with a very high mortality because of the aggressiveness of these tumours.

Epidemiological information: There is growing evidence of the increased incidence of large bowel cancer with the adoption of western ways of life, the diet in particular. An example is the steady rise in the incidence of colorectal cancer in Puerto Rico, where an American type diet is becoming increasingly popular. Another example is the Japanese migrants to Hawaii and California, who have departed from the low incidence characteristics of the Japanese and within a generation have caught up with the local incidence of the host population. Adenomatous polyps have a geographic incidence and distribution similar to that of bowel cancer. In fact they tend to occur concurrently in the same patient. This association suggests related causative factors. (Haenszel et al 1973)

Dietary information: Dietary change as a probable causative factor has been strongly suggested by the following observations.

1. Diet is the most important factor affecting the environment of the colonic mucosa. (Burkitt 1975)
2. Diet alters the bile acid content of the faeces which is believed to be cocarcinogenic. (Reddy 1973, 1981)
3. Colon cancer is closely related to highly processed foods being rare in less well developed countries. (Burkitt 1971)
4. Colon cancer is rare in all animals who have a high roughage content in their diet.
5. The gastrointestinal canal and the bronchi are the only epithelial lined ducts prone to cancer formation, and they are the only ones in which we can alter their contents.
6. Diversion of the faecal stream in experimental animal models for colon cancer reduces the contact time of faeces with the colonic musosa, and reduces the incidence of tumour induction distally. (Campbell et al 1975)
7. Dietary bulk decreases the intestinal transit time and reduces the contact time of the faeces with colonic mucosa. (Cummings 1976, 1978*)
8. Left sided colonic lesions are commoner as the bowel contents become more concentrated, as water is reabsorbed and the stools are stored on the left side of the colon. (Martin 1982*)
9. Potential environmental carcinogens have been isolated in the food we eat, and the breakdown products of some of the compounds in the preserve industry are similar to those used in the experimental model for colorectal cancer induction. (Weisburger 1982*)

Dietary fibre information. The possible relationship between colon cancer and fibre deficiency in the diet is defined below.

1. The colonic physiology is altered by the intraluminal contents, and a close relationship has been demonstrated between the unabsorbable cellulose content of the diet and bowel behaviour. A diet rich in unabsorbable roughage passes more rapidly through the bowel and produces large soft stools with little odour.
2. Any potential carcinogen in a constipated colon will not only be concentrated in the reduced faecal mass, but will also be longer in contact with the colonic bowel mucosa and both these factors could enhance the carcinogenicity of any noxious bowel contents.

Colonic bacterial flora. The following observed facts suggest that the bacteria present in the colonic environment may be indirectly involved in the induction of the bowel cancer.

1. The greatest incidence of bowel cancer in man is in the left colon where bacterial proliferation is maximal. (Burkitt 1975)
2. Tumours are rare in the small intestine which has rapidly passing and relatively abacterial contents. (Burkitt 1975)
3. Carcinogens which produce intestinal tumours in rats, are ineffective if the rats are kept in a sterile environment with resulting bacteria free faeces. (Wiebecke 1969)
4. Western diets alter the bacterial flora of the intestinal content and epidemiologically bowel tumours are related to a western diet. (Hill 1971)
5. Bacteria can cause the degredation of normal bile salts to form known carcinogens. (Reddy 1981*)

6. Differences between the bacteria in the faeces of Africans (a low risk group) and Europeans (a high risk group) have been demonstrated. (Burkitt 1974)
7. Bile acid excretion is altered by the diet, and it is increased in the high risk groups. (Domellof et al 1982*)

Alteration in the diet to the more highly processed foods of economically well developed communities, alters the colonic environment. Bile acid degredation may lead to the formation of co-carcinogens, some being tumour promoters in experimental work. In a cellulose depleted diet and the resultant colonic stasis, such co-carcinogens in the smaller faecal mass would not only be present at a higher concentration, but would also be in contact with the colonic mucosa for a prolonged period of time which could induce malignant changes in a area of colonic epithelial dysplasia (Illustration 7).

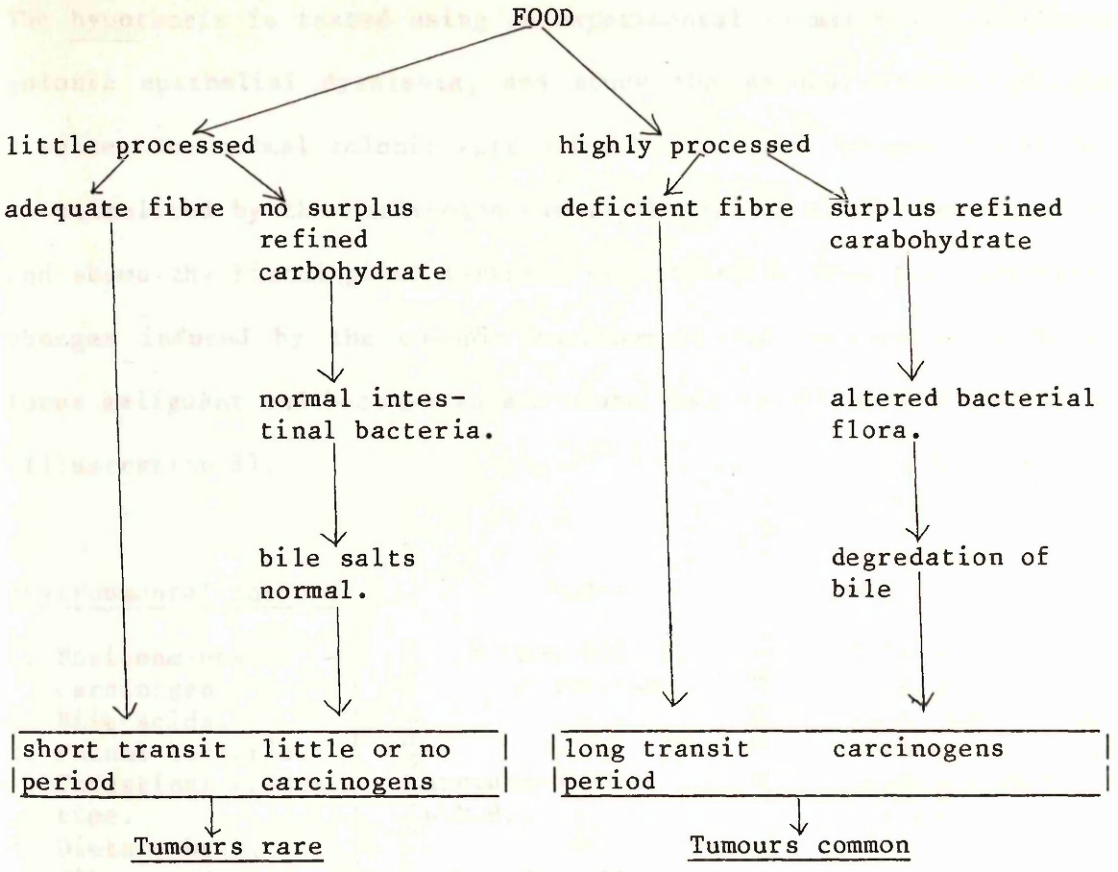


Illustration 7

Flow diagram of the possible relationship between diet and cancer of the colon (Burkitt 1971)

The hypothesis is tested using an experimental animal model to induce colonic epithelial dysplasia, and study the natural history of the disease. The normal colonic epithelium in the male Sprague Dawley rat is stimulated by the carcinogen Dimethylhydrazine to become unstable, and shows the histological features of dysplasia. This then undergoes changes induced by the colonic environment and becomes an adenoma, turns malignant and becomes an adenocarcinoma and finally metastasizes (Illustration 8).

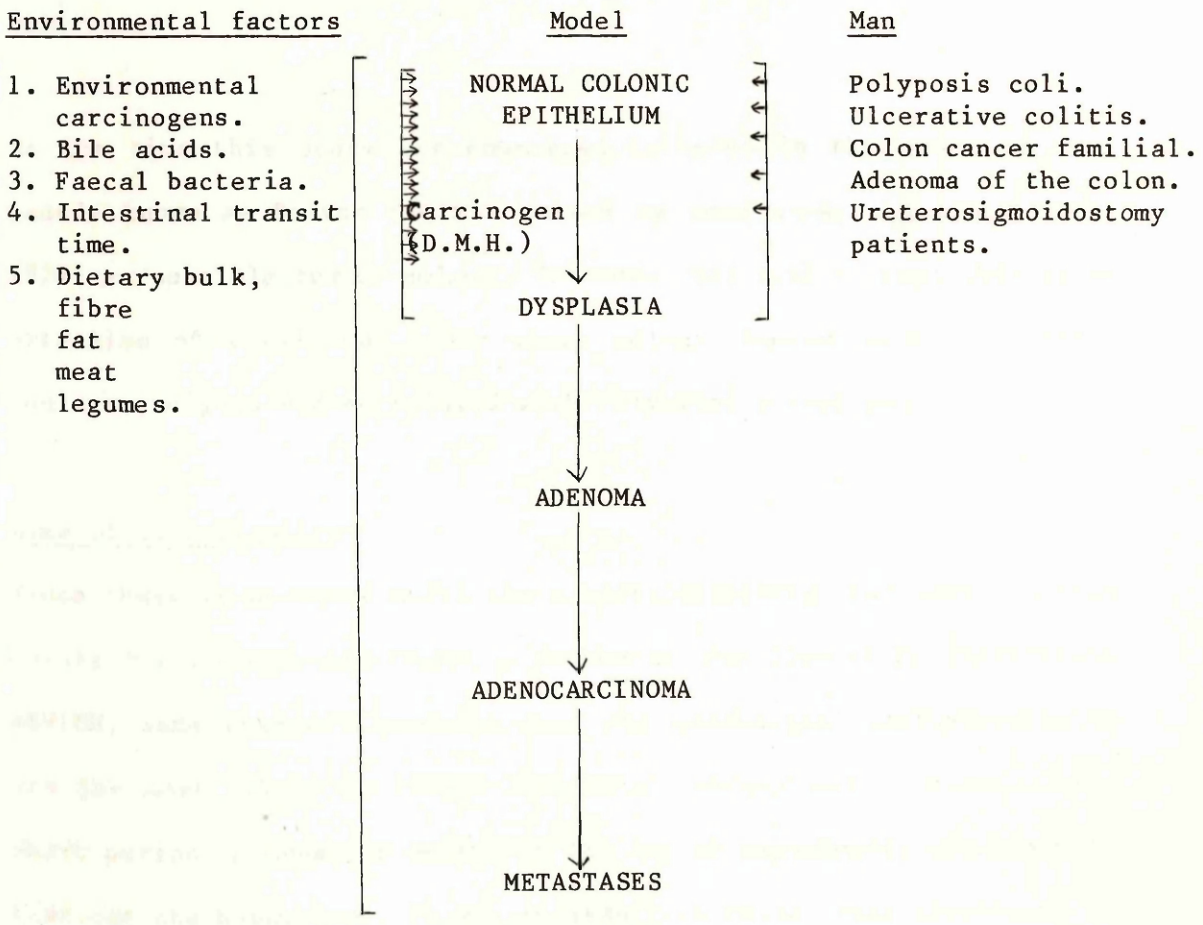


Illustration 8
Hypothesis

IV. Application and aims of investigation.

Dietary habits, environmental carcinogens as well as endogenous carcinogens have been implicated in the aetiology of multiple polyps and colonic neoplasia in man. Those conditions that are well known to predispose to colonic neoplasia all have an unstable colonic mucosa, which develops areas of colonic dysplasia. The tumours that develop are multiple synchronous and very malignant. Any attempt to test these hypotheses of their aetiology requires a reliable animal model for colonic dysplasia in which strict controlled studies can be carried out.

At the time this study was commenced interest in the group of compounds known as Polyamines was raised by some workers (Russel et al 1971) as possible tumour makers. The model was used to test this as an extension of a clinical study where colonic tumour mass in a model could be weighed and correlated with Polyamine secretion.

Aims of investigation.

Since there is no known model for colonic dysplasia, but many reported models for colonic carcinoma, a review of the literature (HISTORICAL REVIEW, same chapter) revealed that the carcinogen Dimethylhydrazine was the most likely to induce colorectal tumours within a relatively short period in order to carry out the set of experiments necessary to test out the hypotheses. It was necessary to repeat this experiment in order to confirm that indeed colonic cancers can be induced by this carcinogen, and this was the aim of the first experiment that was carried out in CHAPTER TWO - THE EXPERIMENTAL MODEL (I). This model was used in an attempt to develop a new model for colonic dysplasia

using the same carcinogen, but reducing the total dosage given, till epithelial dysplasia was seen in the colon. These areas of dysplasia were then allowed to develop without any further stimulation from the carcinogen into Polypoidal cancers. This aim was achieved in CHAPTER THREE - THE EXPERIMENTAL ANIMAL MODEL MODIFIED FOR COLONIC DYSPLASIA (II). In an attempt to alter the natural history of the disease in this model, the design of the next set of experiments was to test the effect of altering single known factors in the colonic environment of the experimental model which could influence the course of the disease.

Alteration of the fibre content in the diet and its effect on the model for colonic dysplasia was the aim studied in the experiment in CHAPTER SIX - DIETARY FIBRE.

The role of the faecal bile acids which are known colon tumour promotors was studied in the experimental model. This was the aim of the experiment in CHAPTER SEVEN - FAECAL BILE ACIDS.

The other factor which could influence the development of colorectal polyps from colonic dysplasia in the model is the duration that intraluminal tumour promotors spend at the area of dysplasia, and this aim was studied in the experiment in CHAPTER EIGHT - INTESTINAL TRANSIT TIME.

The last factor studied was related to the dietary fibre which is physically and chemically active in the colon, producing volatile fatty acids, absorbing water and generally increasing the bulk of the faeces. This aim was studied in the experiment outlined in CHAPTER NINE - FAECAL RESIDUE.

Finally the role of Polyamines spermine, spermidine, and putrescine

was evaluated in this experimental model as an extension of a clinical review of their value as tumour makers. This was the aim of the experiment in CHAPTER ELEVEN - POLYAMINES AS TUMOUR MAKERS IN THE EXPERIMENTAL ANIMAL MODEL (II).

CHAPTER ONE. GENERAL METHODS

The general methodology of the procedures used in the majority of the experiments in this thesis are presented in this chapter. The special methods used in the different groups of experiments will be described in the respective chapters.

I. Animals

Male albino Sprague-Dawley rats, weighing between 150 grams and 200 grams were obtained from a random inbred colony. They were housed in groups of four in large wire cages and unless otherwise stated were fed and maintained on a standard diet being allowed food and water (ad libitum). The diet was made up of Oxoid 41 without antibiotics. The only exception to the food being allowed (ad libitum) was when metabolic experiments were carried out and in these instances the food was weighed and returned in the next feed if any was left over. All the animals were allowed to settle down for a week in their new environment before the experiments started. Those rats who looked unwell or who failed to put on weight were excluded from any of the experiments and were replaced with healthy rats of the same weight.

II. Anaesthesia

No anaesthetic agent was used throughout these experiments for injecting the animals with carcinogen. Halothane anaesthesia was used on one occasion (CHAPTER EIGHT) to insert the nasogastic tubes in the

animals in preparation of measuring intestinal transit time. The animals were killed by ether anaesthesia in an ether glass chamber at the termination of the experiments or if the animals did not look well.

III. Operative Procedures

Protective clothing was worn when handling the carcinogen especially in its preparation, since fresh stock had to be made up each week for the first 16 weeks of the experiment. These protective measures were also taken when handling the experimental animals for weighing, cleaning the cages, handling the excreta and injecting the animals. Since it was felt that the greatest hazard was in injecting the animals with carcinogen, special perspex stands were constructed in the shape of a tunnel with a window cut out of the roof. This allowed easy access to the dorsal skin of the rat and so the subcutaneous injections could be done with little effort and maximum precaution. For the metabolic experiments carried out in this thesis, a special cage was constructed to serve our needs (Illustration 9).

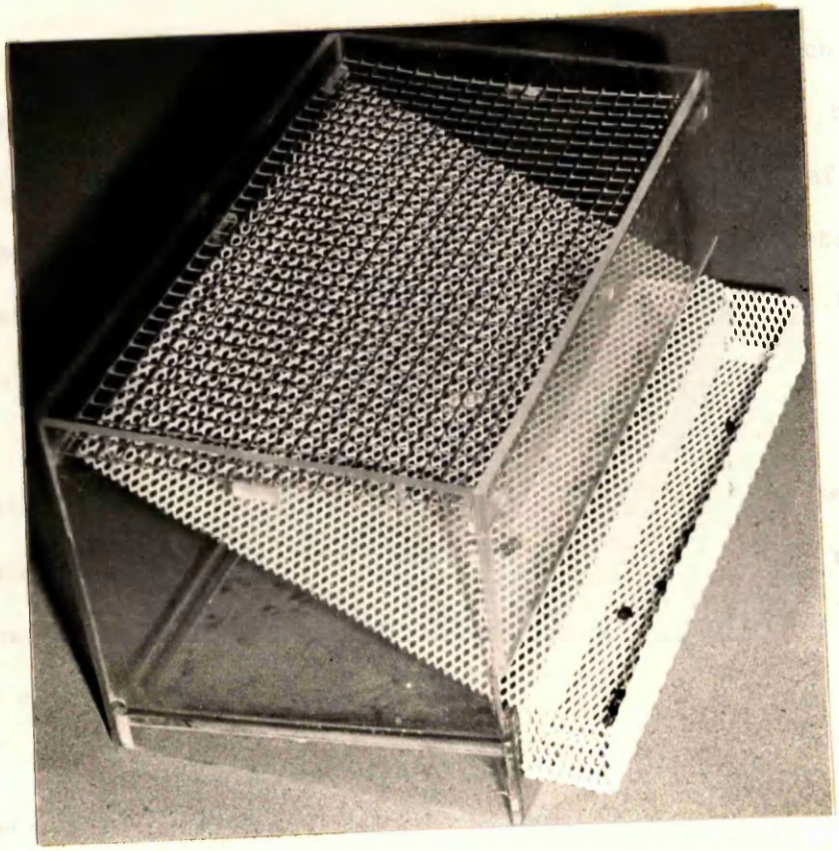


Illustration 9

The metabolic cages designed by the author were constructed so that it was possible to separate the faeces which rolled down an inclining grid to a collecting box. The base has an inclination diametrically opposite which allows the collection of urine free of faecal contamination and is drained out of a tap in the corner of the metabolic collecting unit. The construction is made out of perspex and a plastic coated metal grid for the separation chamber.

The carcinogen used was 1:2 Dimethylhydrazine (DMH) in all the experiments in this thesis. The chemical was purchased in a crystalline form from the Aldrich Chemical Company Ltd.

DMH was dissolved, immediately prior to injection each week, in demineralised water at a concentration of 16.6 mg per ml, then it was neutralised at a pH 6 by sodium hydrogen carbonate, after adding ethylene diaminotetracetate (EDTA) (0.5 mg per ml) for stabilisation as a carrier. This latter substance was used to inject all the control animals in the experiments in this thesis.

The rats were given weekly subcutaneous injections on the dorsum of the animals, a dose of 15 milligrams per kilogram body weight. The doses were calculated to the DMH base (0.45 milligrams for 1 milligram of DMH dihydrochloride).

The rats were weighed weekly at the same time of the morning and a daily watch was kept for their general state of health.

IV. Recordings

In the initial experiment in order to record the animal toxicity from the carcinogen, the stools were tested for occult blood and the haemocrit and liver function tests were performed on a retro-orbital venous blood sample.

Following a full autopsy, the whole of the colon was opened out and pinned on to cork boards to facilitate examination. The number and

anatomical locations of the lesions discovered were drawn on to a diagram for easier documentation. Their appearances were classified as plaques, sessile tumours or polypoidal in shape. The tumours were individually dissected off the colon and weighed using a microbalance to measure the tumour mass.

Light microscopy of the lesions was performed after hematoxylin and eosin staining of the sections. In some instances histochemical staining was used to determine whether the tumours were mucosecreting. The tumours were graded as either well-differentiated or anaplastic adenocarcinomas. The degree of tumour invasion was staged into either lamina propria involvement, invasion of the sub-mucosa or serosa. The regional lymph nodes were also examined after dissection for evidence of tumour metastases and lymphocytic response. Histology was also obtained of the whole of the gastrointestinal tract, liver, spleen and kidney from random sections to detect any histological changes caused by the toxicity of the carcinogens.

V. Calculations

Calculations for making stock solution:-

The stock solution was made from the crystalline form each week and the exact quantity needed was made to avoid carcinogen disposal problems.

1. The doses were weight-related and had to be calculated to the DMH base which was 0.45 milligrams equivalent of one milligram of dimethylhydrazine dihydrochloride. The dose was 15 milligrams of

DMH per kilogram rat body weight. This converted to 0.015 milligrams per gram rat body weight of DMH or the equivalent 0.015/0.45 = 0.0334 milligrams per gram rat body weight of DMH dihydrochloride.

2. Concentration required.

16.6 mg/ml DMH Cl_2

n = number of experimental rats

Xgm = weight of each rat

0.0334 x X x n = 0.0334Xn mg DMH Cl_2 weighed

= 0.0334Xn/16.6 cc of demineralised water required

$= \frac{0.0334Xn \text{ ml}}{16.6} \times \frac{0.5 \text{ mg}}{\text{ml}} = \frac{0.0334Xn\ 0.5}{16.6}$ = mg of EDTA was added to water to stabilise the stock solution and act as a carrier substance.

3. Concentration of DMH

0.45 mg DMH = 1.0 mg DMH Cl_2

? = 16.6 mg/ml

$\frac{0.45 \times 16.6}{1}$ = 7.47 mg DMH/ml

Millilitres of carcinogen per rat injected

X gm x 0.015 = 0.015X mg DMH/rat

$\frac{0.015X \text{ mg} \times \text{ml}}{7.47 \text{ mg}} = \frac{0.015X \text{ ml rat}}{7.47}$

= 0.002X ml/rat.

CHAPTER TWO. THE EXPERIMENTAL ANIMAL MODEL (I)

The experiment described in this chapter was performed to test the reliability of an experimental model for cancer of the colon and rectum, intestinal carcinoma induced in the rat by 1,2-Dimethylhydrazine described by Martin et al 1973. The aim was to modify the model as a model for colonic dysplasia, instead of one for colonic cancer, which was the design of the experiment in CHAPTER THREE.

Any attempt to test the hypothesis on the aetiology of colonic polyps and colonic neoplasia requires a reliable animal model in which strict controlled studies can be carried out. The progress in the experimental induction of colorectal neoplasia has been delayed in the past by lack of specificity of carcinogens to induce colonic tumours. Gastrointestinal tumours have been induced in the rat following the intraperitoneal injections of methylazoxymethanol, an extract of Cycad nut (Laqueur 1965). The symmetrical dialkylhydrazines were subsequently synthesised and it was shown that these compounds were effective gastrointestinal carcinogens in inbred rats (Druckrey 1967).

I. Materials and method

(a) Animals

Twenty inbred rats from Sprague-Dawley were used in this experiment in an attempt to reproduce colorectal cancer. The rats were divided into two groups on arrival and were housed in large wire cages in groups of three or four. The rats of one group were to receive carcinogen, while

the other group were to serve as the controls. Their weight was between 150 grams to 200 grams. They were allowed to settle down in their new cages, and were fed Oxoid 41 diet and allowed to drink water (ad libitum). Unfortunately this first group of animals was unhealthy and experiments were delayed till a fresh group of animals was obtained, ones which looked healthy and were gaining weight.

(b) Operative Procedure and measurements

Dimethylhydrazine dihydrochloride was dissolved prior to injection and was freshly prepared as described in CHAPTER ONE - GENERAL METHODS. The rats in the experimental group were given weekly subcutaneous injections of 15 milligrams per kilogram body weight. The injections were discontinued after 28 weeks of treatment, and a total dose of 420 milligrams per kilogram body weight was given.

The rats were weighed weekly prior to the injections, two of the experimental animals were found dead and the remaining 18 animals were sacrificed at the end of 30 weeks by ether anaesthesia. Gross and histological examination of the abdominal viscera was carried out in each animal. The ten control animals received an injection of the carrier substance EDTA each week at the same time as the experimental animals received the carcinogen, and for the same duration of 28 weeks.

(c) Recordings

Rat No.	Right colon	Left colon	Rectum	No.	Tumour Weight mg.	Ear	Small Intestine
1	3	5	1	9	1.89	0	0
2	2	8	2	12	3.60	T	0
3	DIED						
4	1	7	0	8	0.89	0	0
5	1	4	2	7	0.28	T	2 duodenal
6	DIED						
7	2	3	1	6	1.83	T	0
8	DIED	1		1		T	0
9	2	2	1	5	2.40		1 duodenal
10	1	3	4	8	1.43	T	0

TABLE III

FREQUENCY AND DISTRIBUTION OF TUMOURS INDUCED BY DMH

Rat No.	Plaque (Dysplasia)	Sessile (Adenoma)	Polypoidal	(adenocarcinoma)
1	1	1	7	
2	0	1	11	
4	0	1	7	
5	1	3	3	2 duodenal tumours
7	1		5	
8	1			
9			5	1 duodenal tumour
10		7	1	

TABLE IV

TYPES OF TUMOURS INDUCED BY DMH

II. Results

Clinical symptoms of intestinal lesions appeared in some rats after 20 weeks. Weight loss, retarded growth, roughness of the fur, diarrhoea, and blood in the faeces. Three of the rats in the experimental group died from their tumours, in two it was impossible to obtain any information, but in the third, a limited post mortem was carried out in order to assess whether there were any tumours induced in the colon. Half of the experimental animals developed ear tumours as well as intestinal tumours. A total of three duodenal tumours were induced, in two rats who also had polypoidal tumours in the colon. These tumours were adenocarcinomas. One rat developed gross fatty changes in the liver at postmortem, and histology showed the presence of a fibrosarcomatous change. Two rats developed intestinal obstructions resulting from intussusceptions of the polypoidal adenocarcinoma of the colon. Intestinal cancers were always carcinomas, which were usually well differentiated adenocarcinomata. Most of the rats developed multiple synchronous tumours, mainly polypoidal, although some were sessile adenomas, and plaques of dysplasia. A total of 56 tumours were induced in 8 rats. The anatomical distribution of these were predominantly left sided, and in particular no tumours were ever found in the caecum. There were 12 tumours induced on the right side of the colon, 33 on the left side, and 11 in the rectum (TABLE III). The gross appearance of the lesions varied: there were three plaques of dysplasia, 13 sessile adenomas, and 40 polypoidal adenocarcinomas (TABLE IV). Histologically these were usually well differentiated adenocarcinomas; but mucoid carcinoma, signet ring carcinoma, anaplastic carcinoma were also observed. Invasiveness of the lamina propria was the criterion used to classify a lesion as a carcinoma, in

many of the cases the lesions involved invasion of all the layers of the bowel wall including the serosa. Many of the rats had evidence of metastases to regional lymph nodes, and more distant ones as well to peritoneum, pleura, and pericardium.

Examples of the gross and microscopic findings are demonstrated in the following pages (Illustrations 10-13).



Illustration 10

A colocolic tumour induced intussusception with transperitoneal spread and metastatic spread of an anaplastic adenocarcinoma, arising from a polypoidal neoplasm induced by DMH at 30 weeks into the experiment. In this particular animal there were metastatic nodules in the pleura, and the pericardium, with a blood stained ascities, and a blood stained pleural and pericardial effusion.

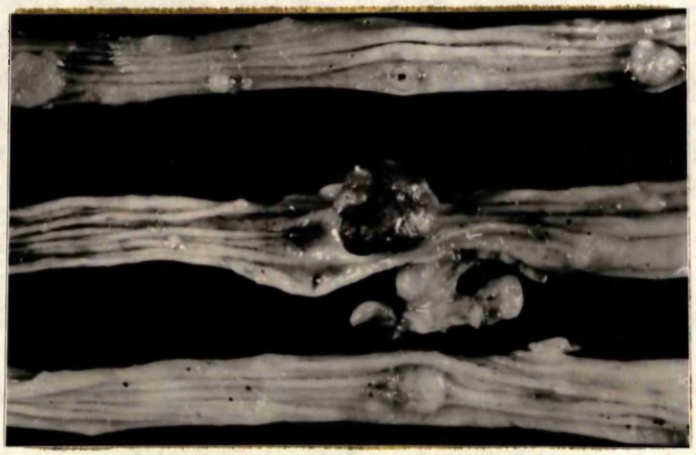


Illustration 11

Macroscopic appearance of the colon of DMH treated rats showing the presence of multiple synchronous lesions. The polypoidal lesion is an adenocarcinoma.

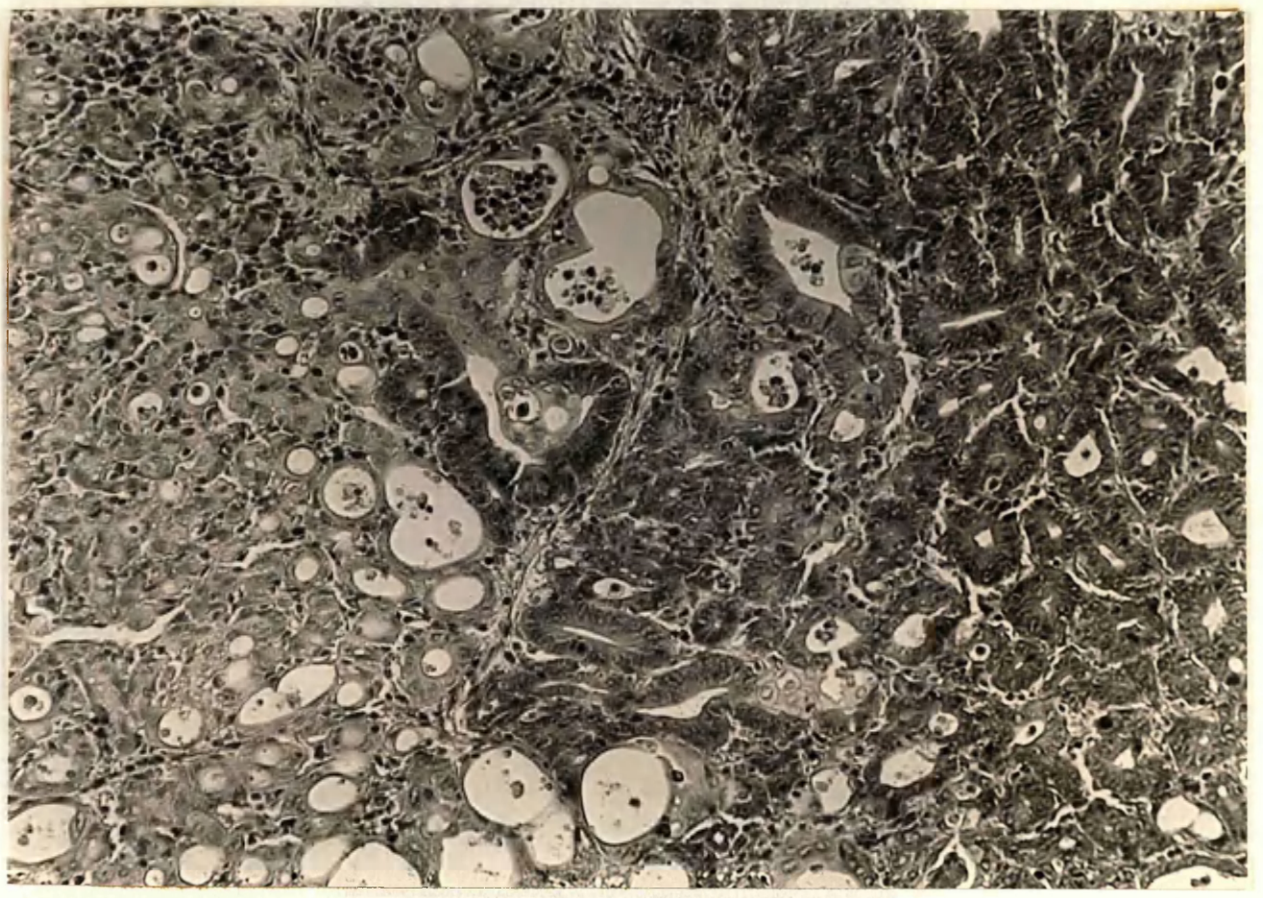


Illustration 12

Histological section of a well differentiated adenocarcinoma invading the stalk of the polyp. The tumour has invaded the lamina propria, and is growing and metastasising towards the lymphatics and the serosal surface to spread transperitoneally.



Illustration 13

This histological section demonstrates the invasion by signet ring anaplastic cells in the colon arising from a polypoidal tumour of the lamina propria of the colonic wall. It is metastasising to the lymphatics and the peritoneal surface for distant spread.

III. Discussion

These results confirm the potency of the carcinogen DMH. It has a selective organotropism for intestinal mucosa, but the ear was another target organ for its toxicity. Carcinoma of the intestine has been induced before, using DMH in inbred BD rats (Druckrey 1970), in random bred Sprague Dawley rats (Schauer 1969) and in random bred Wistar rats (Wittig et al 1971). In all these experiments the incidence of cancer reached 100 per cent when a sufficient dose of DMH was given. The carcinogenic effect was not restricted to the rat but also to the hamster (Osswald and Kruger 1969) in mice, (Lohrs et al 1969). Another reason for the superiority of DMH is its specificity for the intestine, although this is not exclusive. (Martin et al 1973). In this study half of the animals developed ear tumours, and another rat had changes of a hepatic fibrosarcoma. Carcinoma and haemangioendothelioma of the liver, carcinoma of the kidney have all been reported in this model when using high doses of DMH. (Druckrey 1967, Lohrs et al 1969, Schauer 1969). With most of the other carcinogenic agents available, such as 2'-dimethyl-4-amino-biphenyl, 3-methyl-2-naphtylamine or N,N'-2,7-fluorenylbisaetaminde, the incidence of intestinal adenocarcinoma is low, and numerous extra intestinal tumours occur (Schauer 1969 and Spjut 1971).

All the intestinal tumours induced by DMH are carcinomas, although other workers have reported intestinal sarcomas in association with carcinomas (Martin et al 1973). The majority of the tumours induced were well differentiated adenocarcinomas not unlike tumours found in human pathology. The different grades of tumour development was also seen, ranging from plaques with dysplasia, carcinoma in situ, invasion

of the lamina propria, invasion in the wall of the bowel, through to the serosa, lymphatic metastases to regional lymph nodes, transperitoneal spread, and haematogenous spread to more distant areas, in the thorax. The invasiveness as well as the metastatic spread with its complications, are very similar to the natural history of colorectal cancer in man. This similarity stops at the multiple synchronous tumours that are induced in all the animals except one. The metabolism of 1,2- dimethylhydrazine and its relation to the development of intestinal malignancies has been investigated extensively (Druckrey 1967). The metabolic pathway as has been outlined in the GENERAL INTRODUCTION CHAPTER involves a metabolic pathway in the liver, where the compound is metabolised to azomethane, azoxymethane, methylazoxymethanol, and methyldiazonium, which is the ultimate alkylating agent liberated in the lumen of the colon, by the microflora there. Since the liver is involved in the activation, it is not surprising that fatty changes, and fibrosaracoma developed in one of our rats, although a higher incidence has been reported (Martin et al 1973). The presence of three duodenal tumours in this experiment confirms the findings in others (Martin et al 1973), and since the carcinogen, or procarcinogen is excreted in the bile this finding is not surprising, although interfers with the model for colorectal cancer.

Hence, the carcinogen used in this model, 1,2-dimethylhydrazine seems to be the best colon carcinogen available at the present time, even though it has disadvantages of inducing duodenal tumours, ear lesions, and fatty changes in the liver with a potential to go on to fibro sarcomatous changes. Since many of these changes are related to the

dose of the carcinogen, an attempt was made to reduce the toxicity of the model, and find the dose of carcinogen necessary to induce areas of dysplasia in the colon, which would undergo changes in the natural environment of the colon, without any further carcinogenic stimulation. This experiment was attempted in the next chapter, CHAPTER THREE - THE EXPERIMENTAL ANIMAL MODEL FOR COLONIC DYSPLASIA (II).

CHAPTER THREE. THE EXPERIMENTAL ANIMAL MODEL FOR COLONIC DYSPLASIA. (II)

Dietary habits and environmental carcinogens have been implicated in the etiology of colorectal cancer in man (Burdette, 1974) and epidemiological surveys confirm the marked variation in geograghic incidence of this type of cancer (Burkitt et al 1972). Any attempt to test these hypotheses requires a reliable animal model in which strict controlled studies can be carried out.

Progress in the experimental induction of colorectal cancer has been delayed in the past by lack of specificity of carcinogens to induce colonic tumours. Gastrointestinal tumours have been induced in the rat following the intraperitoneal injections of methylazoxymethanol, an extract from the cycad nut (Deschner 1974). Druckrey et al 1967 subsequently synthesized the symmetrical dialkylhydrazine and showed that these compounds were effective gastrointestinal carcinogens in inbred rats. The purpose of this study was twofold: (a) to assess the morphology and histopathology of a new experimental method of inducing colorectal tumours in a laboratory animal by injecting a total dose of carcinogen which induced only dysplastic changes in the colon, the carcinogen being withdrawn subsequently and the progress of these dysplastic changes observed; (b) to reduce the toxicity and side effects of the carcinogen reported in other models.

I. Materials and Methods

(a) Animals

Male albino Sprague-Dawley rats weighing 200 grams were obtained from a random inbred colony. They were housed in groups of four in large wire cages and maintained on a standard diet (Oxoid 41 without antibiotics). The 1,2-Dimethylhydrazine dihydrochloride (DMH) purchased in crystalline form from Aldrich Chemical Company Ltd., was freshly prepared as a solution when needed. Protective clothing was worn while handling the carcinogen, rats, and their excreta. The crystalline DMH was dissolved in sterile water at a concentration of 16.6 mg/ml with 0.5 mg/ml of ethylenediaminotetraacetate added as a carrier and stabilizer of the solution. The 20 control rats received dorsal subcutaneous injections of the carrier alone, and the 40 experimental rats were given weekly dorsal subcutaneous injections of DMH in the dose of 15 mg/kg body weight. The doses were calculated to the DMH base (0.45 mg for 1 mg DMH HCl). The injections were discontinued in the surviving rats after 16 weeks. Prior to each weekly injection, the rats were carefully examined, stools tested for occult blood, and the hematocrit and liver function tests performed on a retroorbital blood sample.

(b) Operative procedure and measurements

Four carcinogen-injected rats and two control rats were killed under ether anaesthesia at 4, 8, 12, 16, 20, 23, 26 and 30 weeks following the initial injections of DMH or if they showed any evidence of ill health. Following a full autopsy, the whole colon was opened and pinned out on cork board to facilitate examination. The number and

anatomical location of the tumours was noted as well as whether they were plaques, sessile or polypoidal in shape. The tumours were individually dissected off the colon and were weighed using a microbalance. These results were expressed as a ratio of the body weight of the animal.

Light microscopy of the lesions was performed after hematoxylin and eosin staining of the sections and the tumours were graded as either well-differentiated or anaplastic adenocarcinomas. The degree of tumour invasion was staged into either lamina propria involvement, invasion of the sub-mucosa or serosa. The regional lymph nodes were also examined after dissection for evidence of tumour metastases and lymphocytic response. Histology was also obained of the whole of the gastrointestinal tract, liver, spleen, and kidney from random sections to detect any histological changes caused by the toxicity of the carcinogen.

(c) Recordings

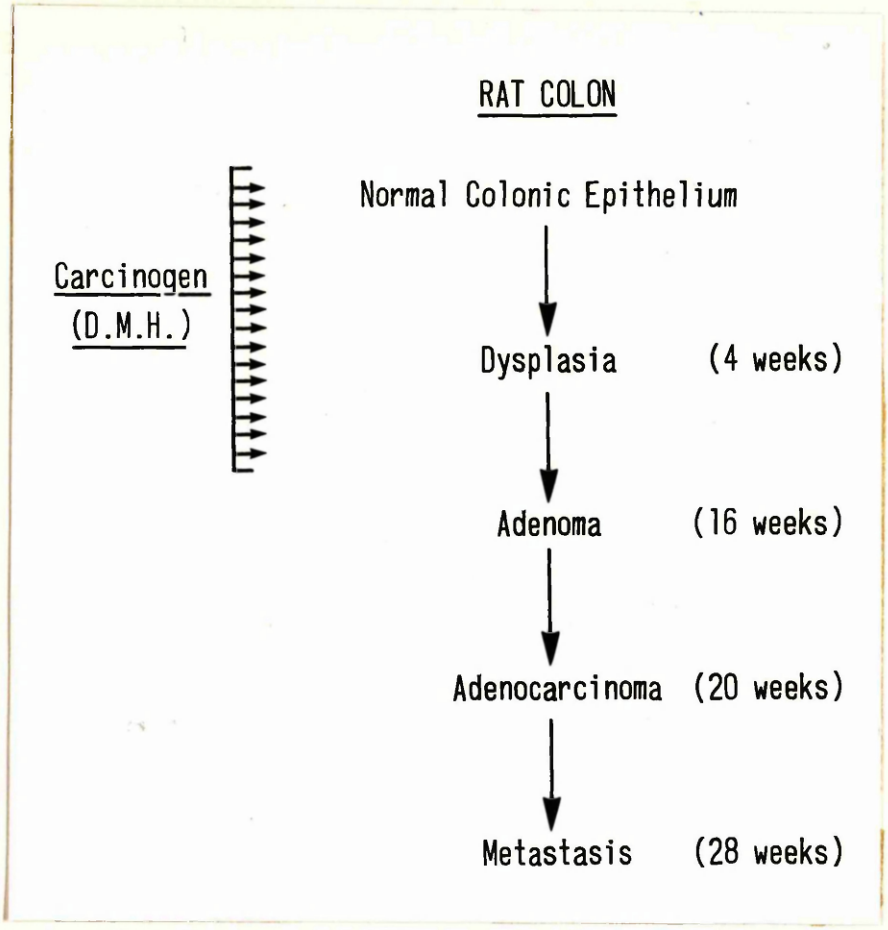


Illustration 14

The time sequence of the development of the polypoidal tumours induced by DMH.

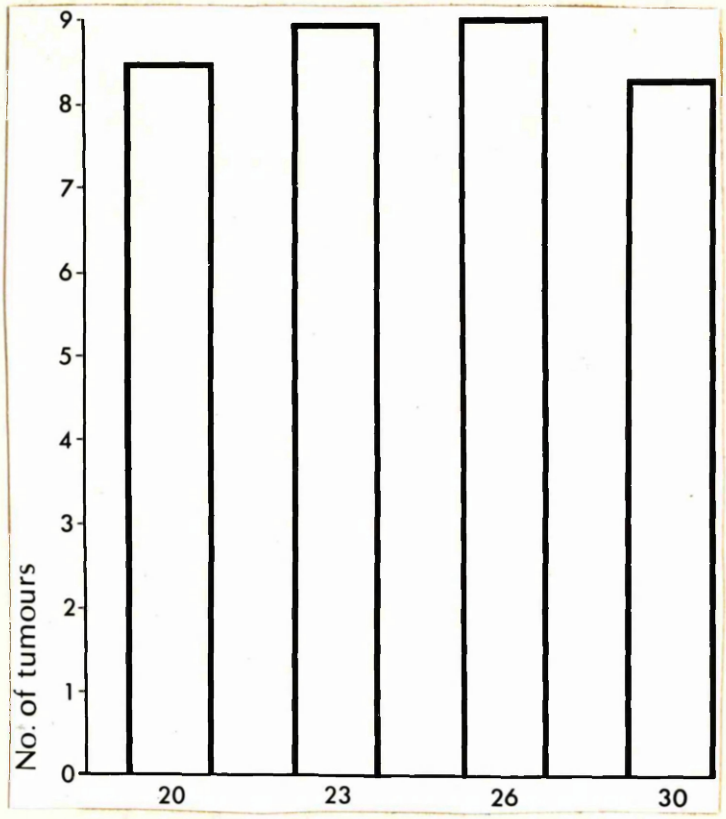


Illustration 15

Histiogram illustrating the total number of tumours induced in each rat during this experiment at four time intervals. An average of 8 neoplasms were induced in each experimental rat. This suggests that for a given dose of DMH these neoplastic areas developed in a constant manner, at sites where the mucosal transformation had occurred, from a stable to an unstable epithelium.

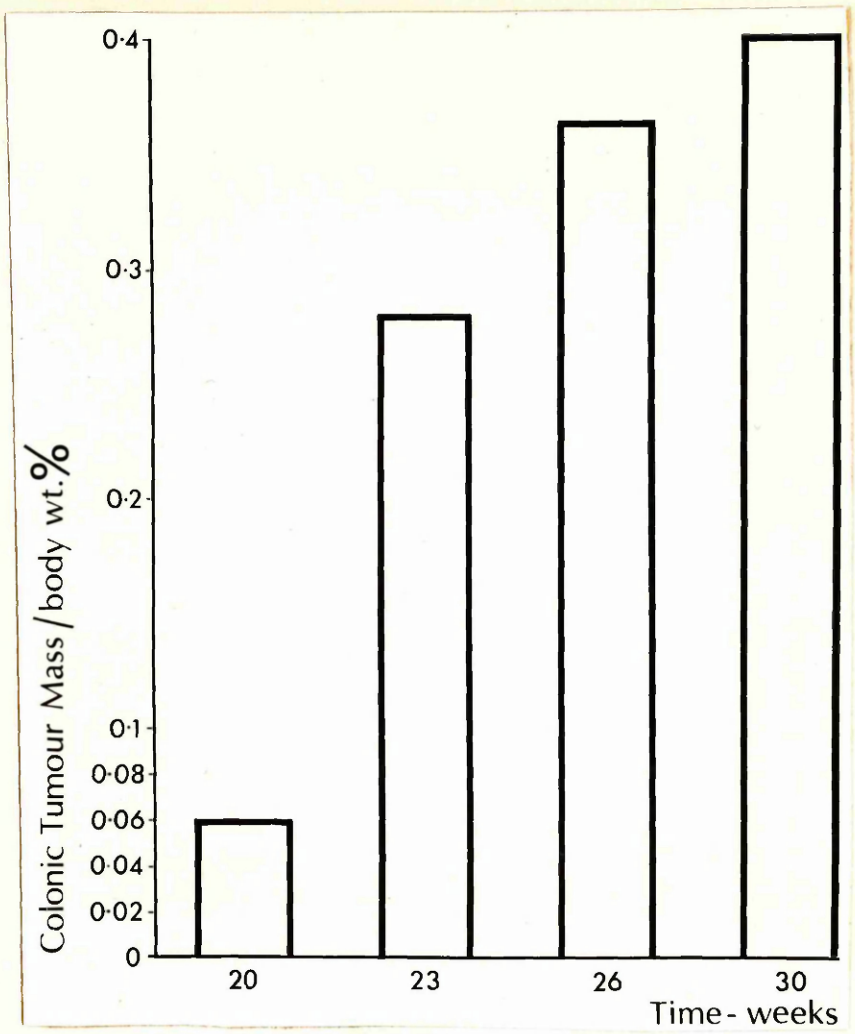


Illustration 16

Histiogram of the ratio of the total colonic tumour mass to the body weight as an expression of the rate of growth of the tumour in relation to the body weight of the rat. The measurements made at 20 weeks, 23 weeks, 26 weeks and 30 weeks illustrate the growth of the tumours from 0.06% to 0.4% within 10 weeks.

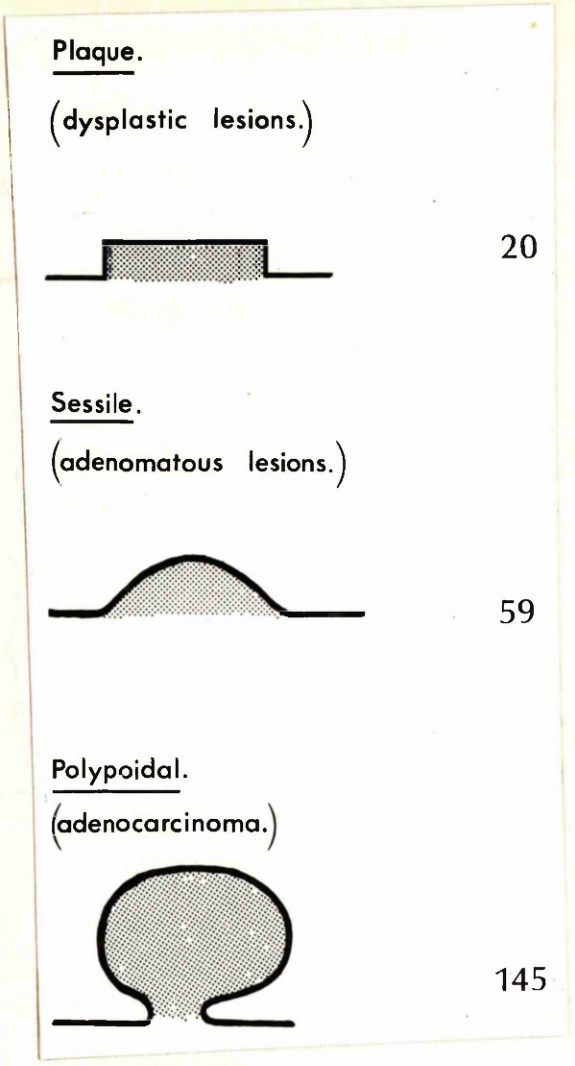


Illustration 17

Macroscopic lesions induced by DMH. The numbers refer to the number of colorectal tumours found in the 25 rats.

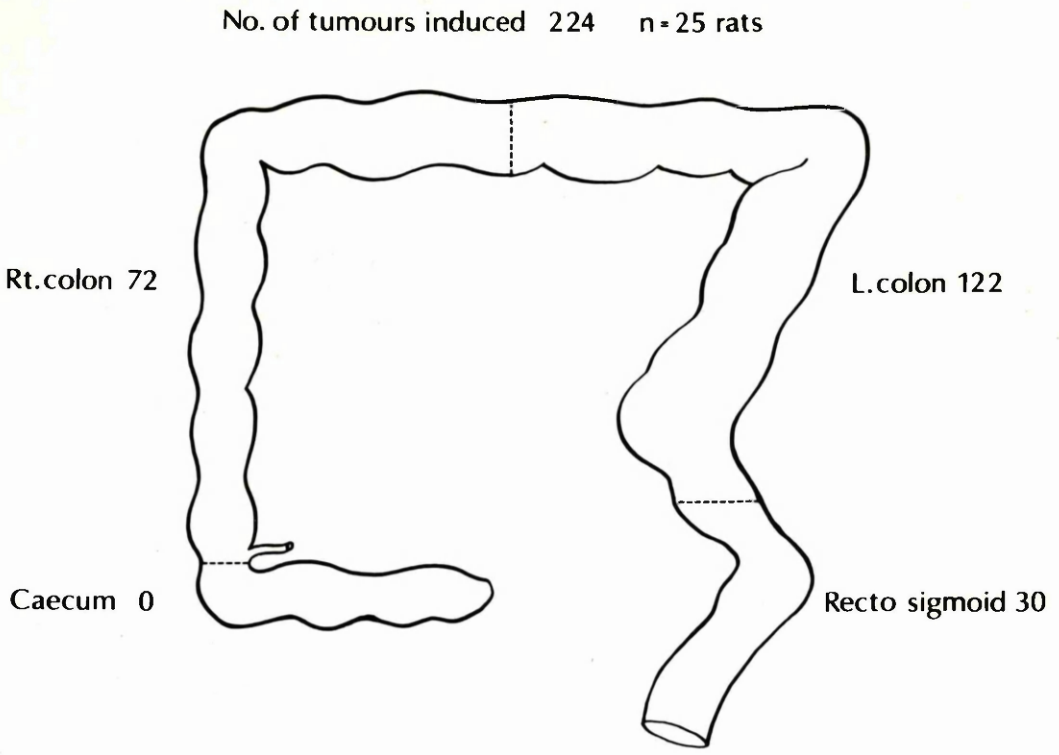


Illustration 18

The anatomical location of 224 neoplasms induced in 25 rats using DMH The lesions induced were predominantly left sided. There were no tumours induced in the caecum.

	Time (Weeks)		
Grading	23	26	30
Well differentiated adenocarcinoma			
Invasion of lamina propria (P1)	8	19	12
Invasion of submucosa (P2)	7	33	18
Invasion of serosa (P3)	3	31	21
TOTAL	18	83	51
%	(86%)	(87%)	(94%)
Anaplastic adenocarcinoma with metastases	3	12	3
%	(14%)	(13%)	(6%)
Number of rats	3	11	6

TABLE V

TISSUE GRADING OF DMH-INDUCED TUMOURS IN THE COLON OF 20 RATS

II. Results

Failure to thrive and weight loss appeared as early as 12 weeks in some rats and other signs of morbidity included roughening of the fur, diarrhoea, blood in the faeces, prolapsing rectum with the terminal obstruction and ascites. Ear tumours appeared in 30% of rats treated with DMH.

Body Weight

This was used to assess the health and well-being of the rats. Control rats progressively gained weight over the 30 weeks but rats injected with DMH failed to increase their weight during the 16 weeks of injections. Once the injections were stopped they rapidly gained weight until the development of intestinal obstruction due to colonic tumours.

Anatomical Location

A total of 224 tumours were induced in the colon and rectum in 25 rats examined. No tumours were found in the caecum; 72 tumours were found on the right side (32%); 122 in the left colon (54.5%), while 30 were found in the rectum and the rectosigmoid (13.5%) (Illustration 18). The mean number of tumours produced in the experimental rats was constant at the four time intervals. The number of colorectal tumours induced in each rat ranged between 8 and 11 and many of these tumours were found in clusters (Illustration 15).

Histopathology

The tumours classified as plaques showed only dysplastic changes, whereas the sessile adenomas histologically ranged from simple adenoma

to carcinoma in situ (Illustration 17). The commonest lesion found was a polypoidal adenocarcinoma. Many of these tumours showed invasion of the stalk which was the cause of intussusception and haemorrhage. A total of 145 polypoidal tumours were present in 25 rats and appeared synchronously in the same rat, often found in groups of two or three tumours in the same site of the colon and rectum.

At 4 weeks after DMH injections, histological sections of the colon showed a marked increse in basophilia in mucosal glands with increased mitosis (Illustration 19). This feature was seen focally throughout the whole colon. No abnormality was found in the control rats. Multiple macroscopic tumours were found along the whole length of the colon at 20 weeks. These appeared to start as sessile plaques of dysplasia which eventually underwent de-differentiation to sessile adenomas (Illustration 20). All the malignant tumours then appeared to arise from these benign polypoidal adenomas with invasion of the stalk of the polyp (Illustration 21). Occasionally the tumours were hyperplastic lymphoid follicles with the tumour arising from the site where the lymphoid follicle encroached on the colonic mucosa. At 16 weeks the lymph nodes draining the site of these lesions were considerably enlarged and adherent to the colon. Reactive hyperplasia was noted without any evidence of tumour metastases.

Histologically the tumours showed a spectrum ranging from simple adenomatous polyps to anaplastic adenocarcinomas. The adenomatous polyps showed changes beginning at the surface (carcinoma in situ) with subsequent invasion of the stalk (Illustrations 22, 23, 24).

At 20 weeks the serosal boundary was invaded by tumour infiltrating on to the peritoneal surface, with metastatic invasion of the regional lymph nodes. Tumour was found to also occur transperitoneally with invasion of the diaphragm into the pleural space and metastases were found in the lungs and the pericardium. At this stage the rats were found to be suffering from the mechanical effects of the tumour. Tumour-induced intussesception was a common finding, as well as blood-stained pleural effusions and ascites (Illustration 14).

Histopathological differentiation and invasion was assessed in 20 rats. The tumours were either well-differentiated or anaplastic adenocarcinoma, most of the latter being mucoid with signet ring formation. The degree of invasion was assessed as to whether only the lamina propria was invaded (Grade P1) or submucosa (Grade P2) or through to serosa (Grade P3) (TABLE V).

The commonest tumour found was a moderately well-differentiated adenocarcinoma. The degree of invasion was time dependent. At 23 weeks, more tumours were P1 than P3, but at 26 and 30 weeks there was a shift to a predominance of P2 and P3 grading with metastases to lymphnodes. Approximately one in eight of the tumours were anaplastic and they showed a more aggressive behaviour and were more advanced in their stage of invasion than the well-differentiated adenocarcinoma. The majority of the tumours that were anaplastic had already metastasised to regional lymph nodes and more distally. Both well-differentiated and anaplastic adenocarcinomas were found simultaneously in the same rats.

Tumour Growth

After dissecting out all the colonic tumour tissue it was weighed dry on a microbalance. The relative growth of the tumour was expressed as a ratio of colonic tumour mass to total body weight. By recording this value at four time intervals it was possible to establish a value of tumour growth in the rats. At 20 weeks the ratio was 0.06% with a sharp fourfold rise in tumour growth at 23 weeks when the ratio was 0.28% of the total body weight. The growth pattern was continued to 0.45% at 30 weeks. The mean weight of the rats was 450 (SD $\pm$ 0.2)g. However, at 30 weeks extracolonic tumour metastases were present and the ratio is therefore much lower as the metastatic tumours could not be weighed (Illustration 16).

Other Features

No tumours were ever found in the injection sites, and none of the rats ever developed liver metastases, although metastatic disease was present in the liver capsule in two rats with transperitoneal tumour spread. Two rats developed small duodenal tumours opposite the ampulla of Vater. A number of the rats developed squamous cell carcinoma tumours of the middle ear, which is another target organ for DMH. At 30 weeks the liver showed some evidence of fatty and other non-specific degenerative changes. All the tumours encountered were carcinomas and no sarcomas were seen in the rats. All control animals receiving the carrier injections were perfectly healthy with a normal histological appearance of the colon, rectum, and liver.



Illustration 19

Histological section of colonic mucosa at 4 weeks showing focal changes in the glands. These multifocal changes were seen throughout the whole extent of the colon at 4 weeks into the experiment. These changes were an increase in mitotic figures, increased basophilia in the crypts of the glands. (Haematoxylin and eosin stain x 150).



Illustration 20

Histological section of a sessile adenoma showing numerous dysplastic changes in the surface at 18 weeks. (H&EX50).



Illustration 21

Adenoma with surface malignant changes at 20 weeks

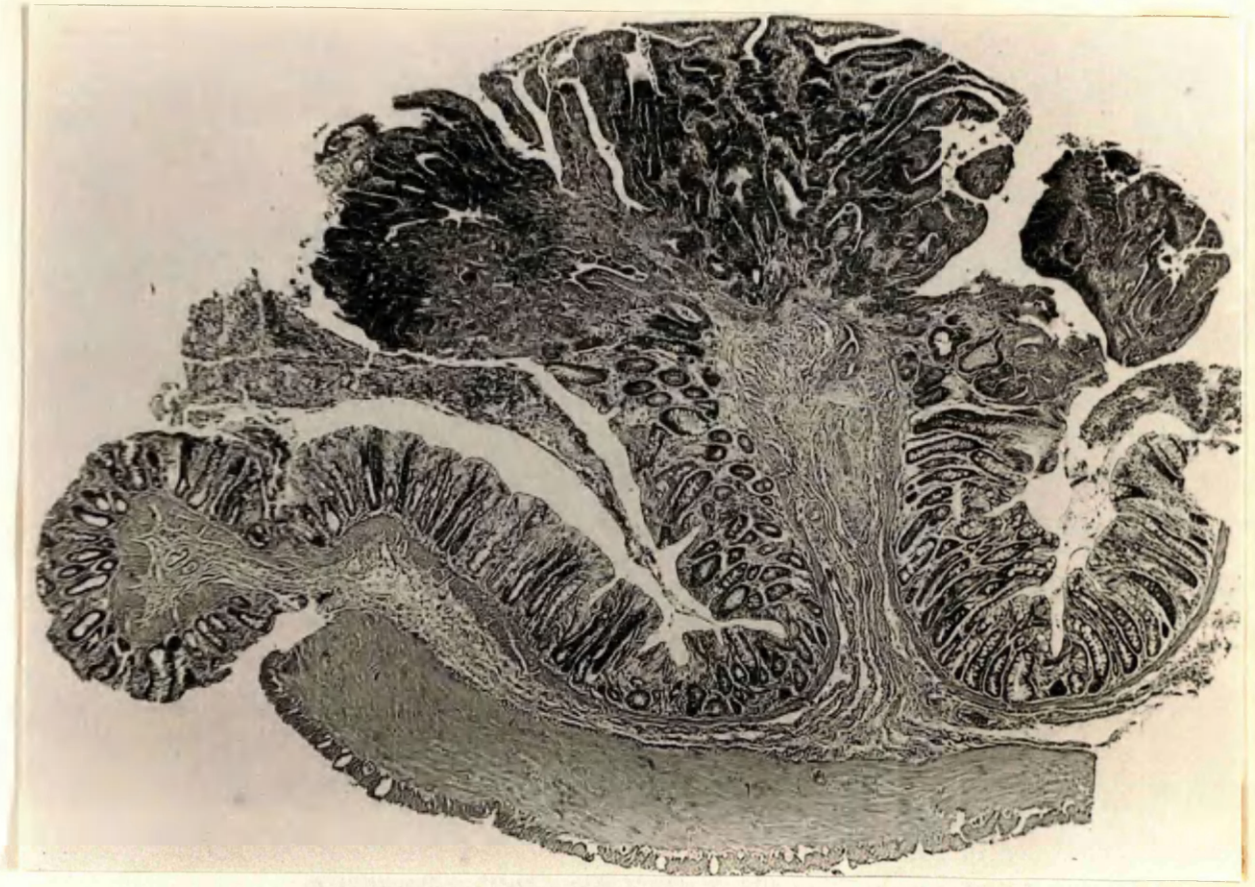


Illustration 22

Polypoidal tumour in crossection, with early carcinomatous change on the surface of the polyp, and invasion of the stalk. The commonest tumour encountered is the well differentiated adenocarcinoma which is seen on this histological section.

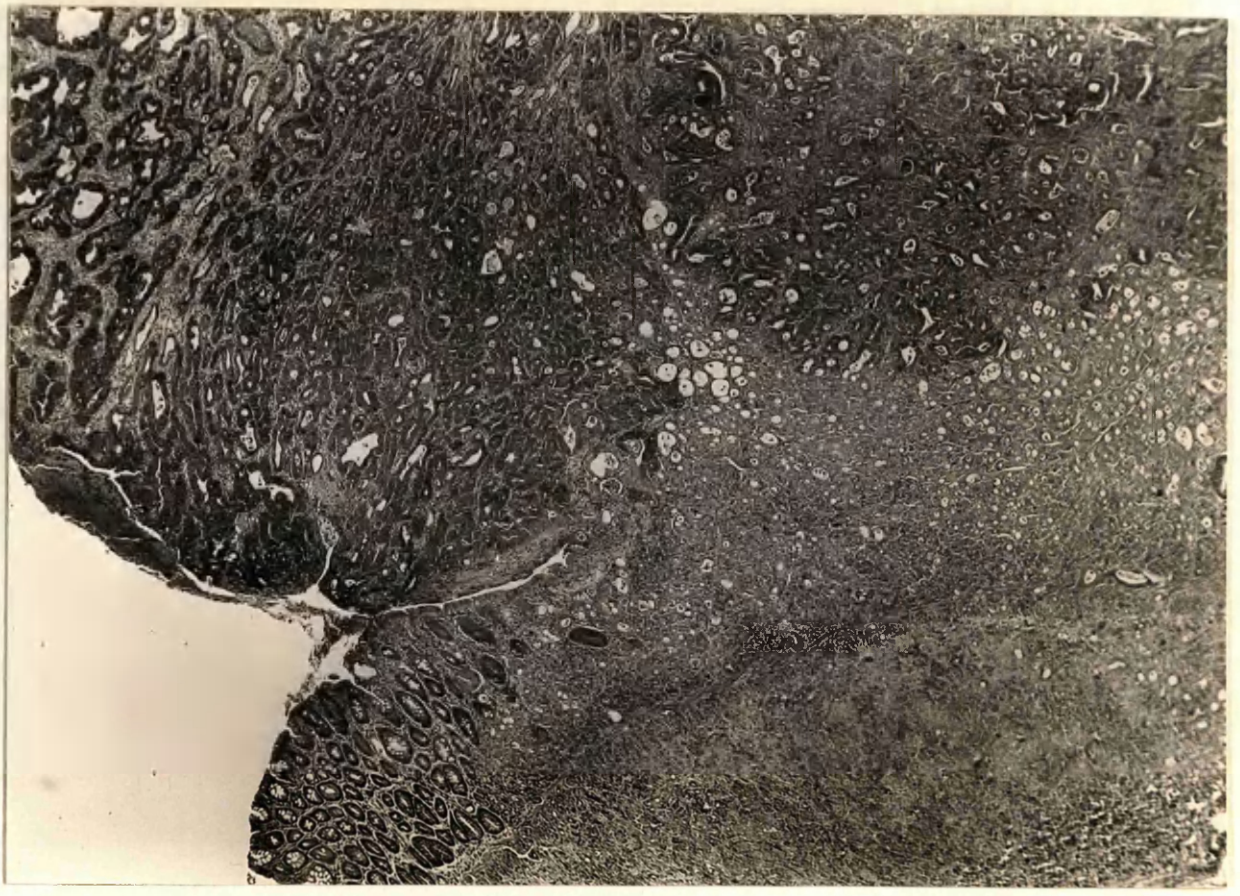


Illustration 23

Histological section of the edge of a tumour induced by DMH (H&EX50). The edge of the stalk of the polypoidal tumour is completely invaded by well differentiated adenocarcinoma which has invaded the adjacent tissues as well. One can see some normal glandular structures which have been spared at the edge of the surface of the polyp.

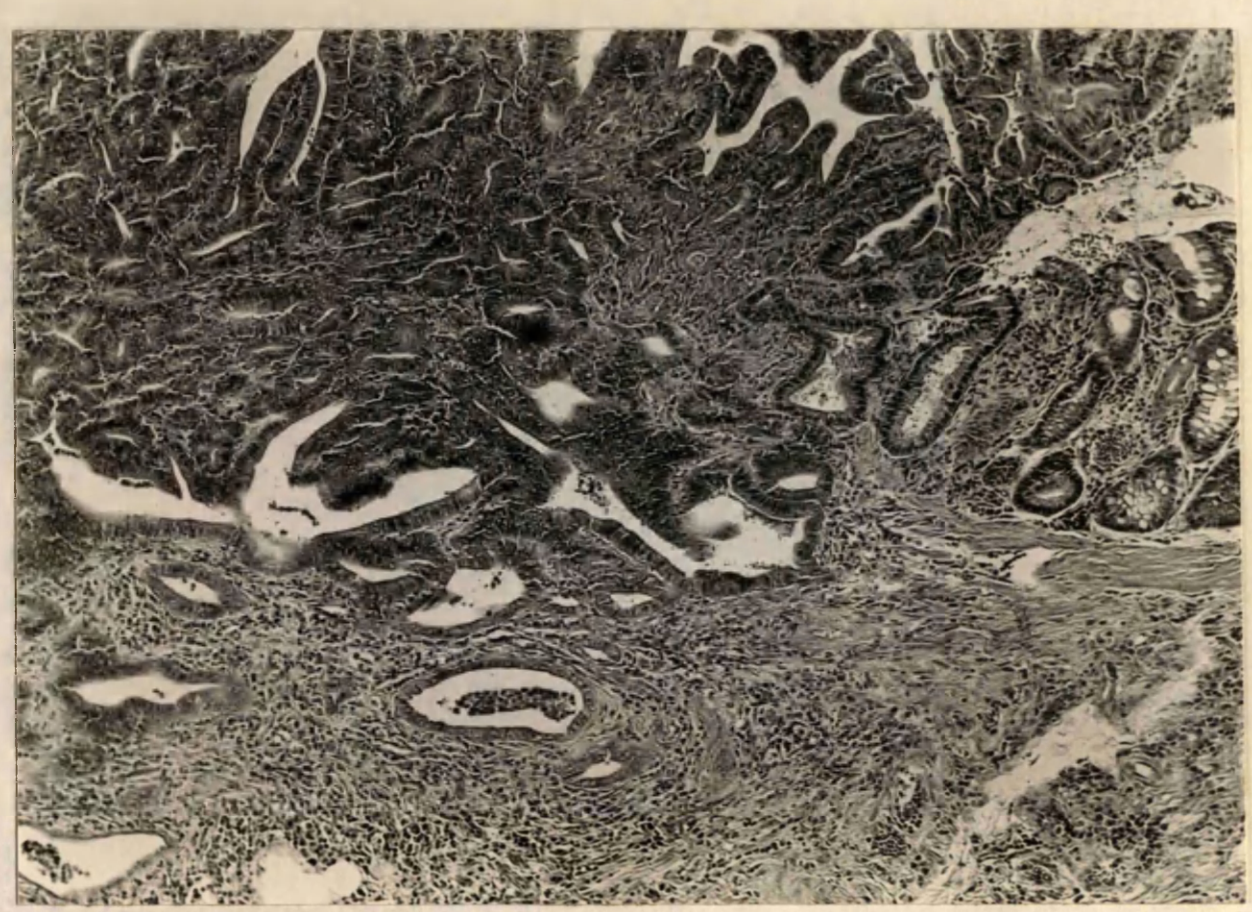


Illustration 24

Invasion of the stalk of a polyp induced by DMH. Well differentiated adenocarcinoma arising from the surface of the polypoidal neoplasm, has metastasised through the lamina and has invaded the stalk. This occurred at 26 weeks into the experiment. There is also a considerable degree of lymphocytes suggesting immunological defence mechanisms attempting to contain the tumour.

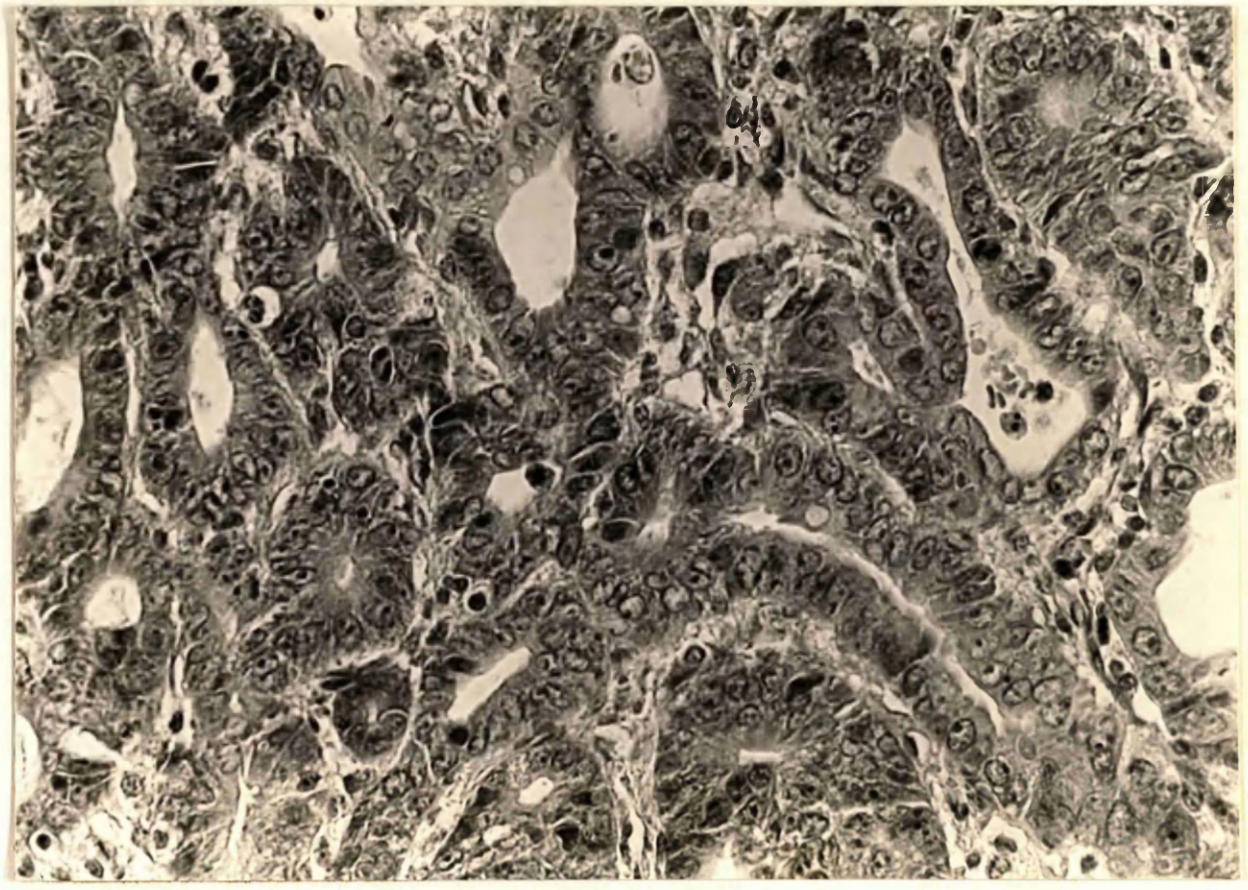


Illustration 25

Histological section of tumour induced by DMH in the rat. (H&EX500). This section shows anaplastic adenocarcinoma with cosinophilic cells present, the significance of which is not known. Large giant cell nuclei with prominent nucleoli and numerous mitotic figures are indications of the aggressive tumour with early metastases.

III. Discussion

The incidence of spontaneous colonic cancer in rats has been reported at 0.1% (Miwa et al 1976, Presean et al 1973). Previous attempts at inducing colorectal cancer in a laboratory animal were unsuccessful due to lack of specificity and organotropism of the carcinogen for colonic mucosa. The discovery by Laqueur 1965, of the carcinogenic effect of cycasin and its aglycone on intestinal mucosa opened the way to the development of new synthetic carcinogens. Druckrey et al 1967 demonstrated that the synthetic dialkylhydrazines were organotropic for gastrointestinal mucosa.

Previous workers have used 4-aminobiphenyl (Walpole 1958, Weisburger 1971). 3'2'-dimethyl-4-aminobiphenyl (Spjut et al 1971), cycasin (Laqueur 1963), dimethylhydrazine (Druckrey 1967), azomethane (Weisburger 1971), N'-methyl- N'-nitro-N-nitrosoguanidine (Narisawa 1971), methylazoxymethanolacetate (Zedeck 1974) and N'-methyl-N-nitrosourea (Narisawa 1975) in an attempt to induce colorectal cancer in an experimental animal.

Tumours have been induced in inbred and random bred rats (Spjut et al 1971), mice (Walpole 1958), guinea pigs (Narisawa 1975), and hamsters (Osswald 1969) by intrarectal instillation (Narisawa 1971), intra-peritoneal injection (Reuber 1976), subcutaneous injections (Deschner 1974), and the oral feeding of these carcinogens (Spjut 1971) as a single loading dose (Zedeck et al 1974) or separate doses given over a period of time (Laqueur 1965). Important factors to consider in tumour induction in an experimental animal include type of carcinogen, mode of administration, dose, and species of animal used. Most of the

carcinogens listed except for dimethylhydrazine and its metabolites azomethane and methylazoxymethane produce a low incidence of intestinal adenocarcinomas and the induction of extraintestinal tumours is common (Spjut 1971, Weisburger 1971, Martin 1982*).

The mode of administration is important since a single injection of methylazoxymethanol (Zedeck et al 1974) produces colonic polyps as well as small bowel tumours whereas an intrarectal infusion of the same carcinogen produced squamous cell carcinomas and myosarcomas (Narisawa 1975). Administration of dimethylhydrazine in the dose of 15 mg/kg body weight for 7 months produced both colonic tumours and small intestine tumours in 60% of the rats with hepatotoxicity and a liver fibrosarcoma (Martin et al 1973). When a total lower dose of methylazoxymethanol was administered intraperitoneally, male rats developed multiple polypoidal carcinomas in the left colon (Reuber 1976).

In our experiment 15 mg/kg of the carcinogen per week was administered as dorsal subcutaneous injections for 16 weeks. A total of 90 mg of DMH was sufficient to produce between 8 to 11 dysplastic areas in the colon in each rat. This model we have described is unique because of the low total dose of DMH given to achieve this objective. The first was the induction of dysplastic colonic lesions alone, which spontaneously underwent changes progressing to adenomatous polyps with subsequent production of carcinoma. The second being the reduction of toxic side effects from the carcinogen to a minimum, without interference with the rate of tumour growth.

It is a reliable and easily reproducible model with a high percentage

of tumour induction within a relatively short period of 18 to 20 weeks.

After a subcutaneous injection, 1,2-dimethylhydrazine hydrochloride enters the blood stream and is then metabolized by the endoplasmic recticulum in the hepatocytes (Laqueur 1965, Weisburger 1971). Hydroxylation in the liver, changes the compound into the bioactive amines, azomethane and azoxymethane. These compounds are then conjugated and excreted as B-glucuronide in the bile (Weisburger 1971). The enzyme B-glucuronidase in the colonic bacterial flora breaks down the metabolite and converts this compound to methylazoxymethanol. In germ-free animals this does not occur and most of the dimethylhydrazine can be collected in the urine and faeces and no tumours develop (Wiebecke 1969). Spontaneous breakdown to methyldiazonium then occurs and the methonium is believed to be the final alkylating agent for carcinogenesis.

The incidence, site of tumour induction, histological features, morphological behaviour and natural history probably resemble the human counterpart of this disease. The histological typing was similar in that both anaplastic as well as differentiated tumours were induced, the latter being the commonest. This tumour model of colorectal cancer is relatively easy to produce and the carcinogen is available and easy to prepare provided the necessary precautions are taken. Only two rats developed small bowel tumours and these were in association with colonic carcinoma. Another unexplained target organ is the ear. Many of these rats developed middle ear tumours which has been previously reported (Wiebecke 1969).

Although wrong to draw too many comparisons to human colonic cancer from this experimental model, it does present many analogous features to colonic carcinoma found in association with polyposis coli in man. Patients with a genetically unstable colonic mucosa who develop multiple adenomatous polyps are at a high risk of developing cancer of the colon. In this model we have demonstrated a preponderance of polypoidal tumours. The total number of tumours induced per rat was found to be relatively constant at various time intervals during the experiment. This can be explained by the initial effect of the carcinogen initiating multiple foci of dysplasia along the colon. Some of these areas are possibly exposed to an environment of bile acids, faecal flora, and dietary compounds which enhance the carcinogens leading to adenomas. Once the stimulus of carcinogen is stopped, no more new tumours are formed, but those that have been induced become autonomous, surface changes occurring with eventual invasion of the lamina propria.

Further studies can now be performed regarding the kinetics of tumour growth, biological markers of tumour cell mass, and the effects of diet and chemotherapy studies on tumour induction.

The search for carcinogens in the external and the colonic environment continues and dimethylhydrazine may well play a role in the etiology of colon cancer. The final breakdown products of DMH metabolism are the same as those of dimethylnitrosamine. This chemical is found in a number of preservatives used especially in the fish and meat canning industry of Japan (Sander et al 1971, Klubes and Jondorf 1971, Hawksworth and Hill 1971).

CHAPTER FOUR. CARCINOGEN TOXICITY IN THE EXPERIMENTAL MODEL FOR COLONIC DYSPLASIA (II).

The carcinogen, Dimethylhydrazine used in this experimental model is given as a total low dose to achieve two objectives. The first objective is to induce areas in the colon with an unstable epithelium which develops into dysplasia. The second objective is to reduce the extracolonic lesions caused by the toxicity of the carcinogen. In an attempt to study the second objective, this experiment was designed to look at the toxicity in this experimental model for colonic dysplasia.

I. Materials and methods

(a) Animals

Male albino Sprague-Dawley rats weighing 200 grams were obtained from a random inbred colony of rats. They were housed in groups of four in large wire cages and maintained on a standard isocaloric diet (Oxoid 41 without antibiotics). Protective clothing was worn throughout the experiment, when handling the rats, the carcinogen and the excreta of the rats when cleaning their cages. Special restraining stands were used when injecting the carcinogen, which was performed on the dorsum of the rats in the subcutaneous space. Forty experimental rats were injected weekly with Dimethylhydrazine, and a further 20 rats were used as controls.

(b) Operative procedure and measurements

Protective clothing was worn when preparing the carcinogen which was done on a weekly basis for the first sixteen weeks of the experiment. The quantity of the carcinogen made was calculated to be sufficient for one set of experiments because the solution was unstable.

The rats were weighed each week, and their general condition noted, as was the site of the injection. Their stools were tested for occult blood, and all haematological tests, i.e. haematocrit, and liver function tests were performed on samples taken by a retro-orbital puncture from the experimental and the control animals. These samples were obtained at 4 weeks, 12 weeks, 20 weeks and 25 weeks during the experiment.

Four carcinogen injected rats and 2 control rats were killed under ether anaesthesia at 4, 8, 12, 16, 20, 23, 26 and 30 weeks following the initial injections of Dimethylhydrazine, or if they showed any evidence of ill health. A full post mortem was carried out on all the rats, taking note of the general condition of the animals at the time of death, and looking for evidence of ear tumours, and tumours at the site where the injections were performed. The internal organs were inspected and histology of the liver and kidneys was carried out using conventional haematoxylin and eosin staining of the sections.

(c) Recordings

Graph of weights

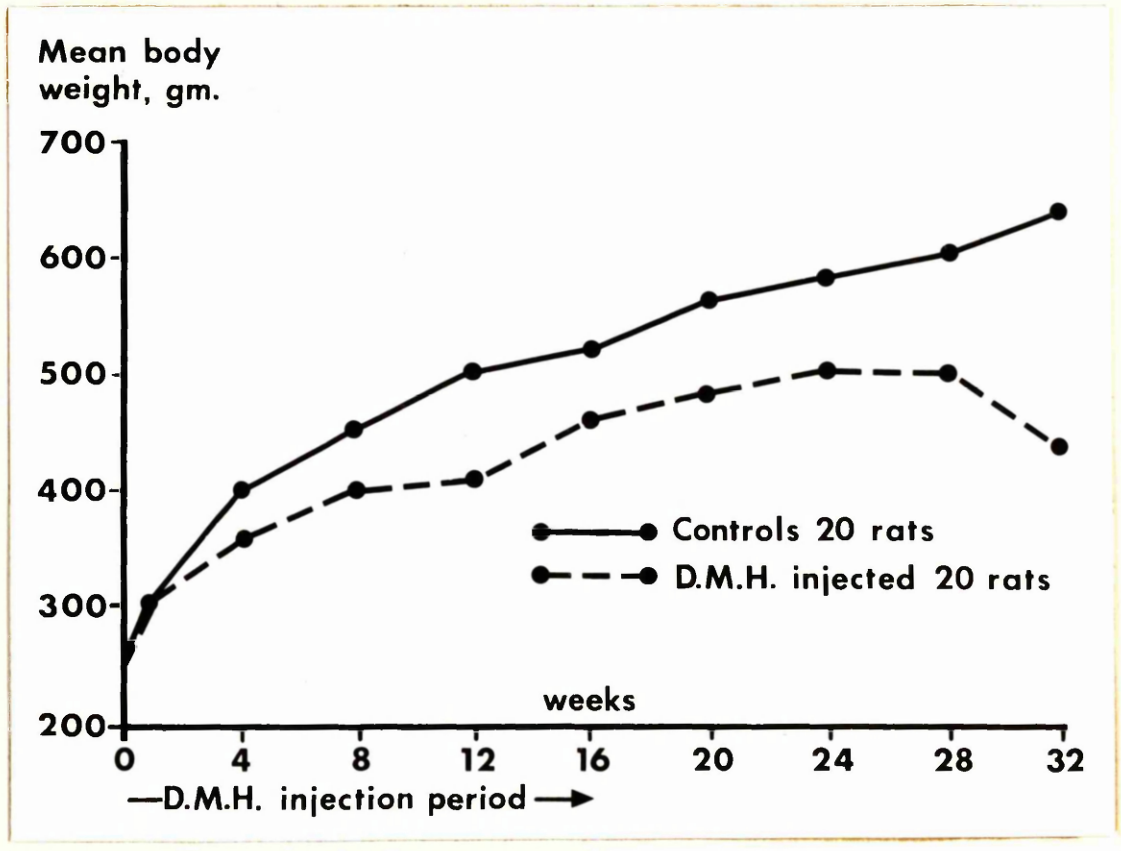


Illustration 26

Body weight of DMH treated animals and controls.

Weeks	Experimental Animals (mean values percentage)								Control Animals (mean values percentage)			
4	50	55	53	51	53.5	49	47	48	51	49.5	-	-
12	43	47	44	46	43	43.5	45	47.5	46.5	48	49	52
20	46	43.5	42	46.5	43	41	42.5	48	50	48	51	53
25	43.5	39	33	40	39.5	36	37.5	42	44	44.5	49	-

TABLE VI

HAEMATOCRIT VALUES

Weeks	Liver Enzymes	Experimental Animals (mean values international units)										Control Animals (mean values international units)				
4	SGOT	117	117	102	90	105	114	117	123	102	123	306	270	120	99	111
	SGPT	66	60	66	54	75	63	63	66	54	52	75	66	48	48	39
	Alkaline Phosphotase	365	378	430	368	479	578	410	430	620	-	66	445	365	365	606
12	SGOT	-	81	-	99	66	117	99	114	108	99	-	102	117	75	87
	SGPT	-	51	-	57	60	69	51	54	60	87	-	36	66	42	51
	Alkaline Phosphotase	-	365	-	245	387	280	-	285	670	701	-	369	440	206	296
20	SGOT	-	123	-	111	114	126	108	114	150	120	-	123	114	132	132
	SGPT	-	117	-	72	225	60	51	90	87	93	-	48	48	48	52
	Alkaline Phosphotase	-	322	-	286	230	275	405	334	510	444	-	378	298	350	330
25	SGOT	-	147	-	174	525	171	213	165	196	213	-	99	168	120	147
	SGPT	-	48	-	45	99	63	48	108	60	48	-	120	75	54	129
	Alkaline Phosphotase	-	330	-	235	260	165	295	350	338	212	-	426	660	580	345

TABLE VII

LIVER BIOCHEMISTRY

II. Results

The Mechanical Effects of Injection of the Carcinogen.

Initially, two of the rats developed ulcers at the site of injection which was on the dorsum of the experimental animals. These ulcers were approximately 0.5 cm. in diameter and healed up rapidly within the week after the use of a polybactrin antibiotic spray applied daily. Another complication at the injection site was the development of a haematoma as a result of trauma from the injection. This subsided and considerably diminished in size although a lump was still felt to be present. This did not appear to bother the animal in any way. When this animal subsequently came to sacrifice at the end of the experiment, histopathology was carried out and showed it to be a calcified haematoma, free of any tumour.

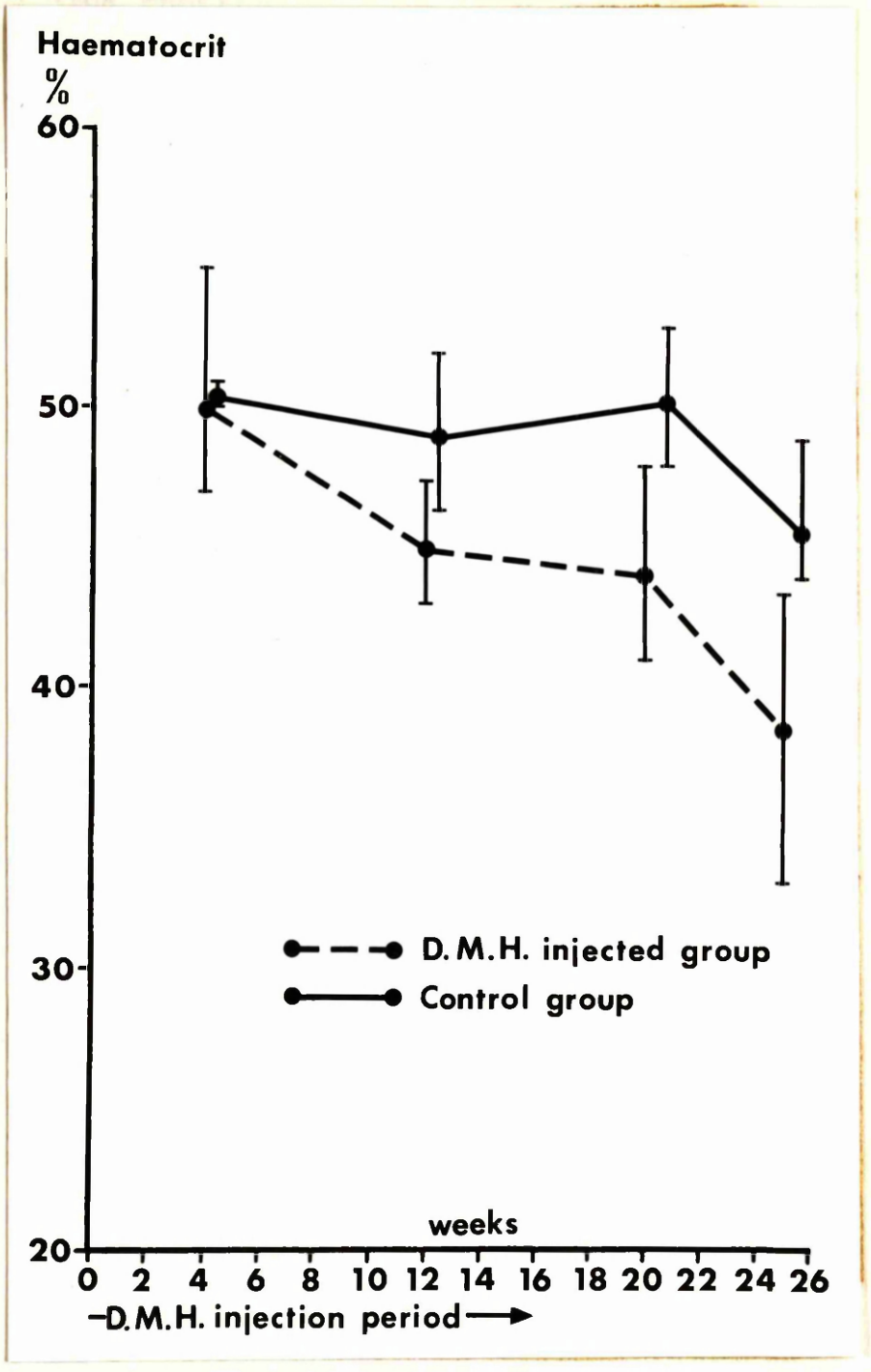


Illustration 27

Haematocrit values of experimental and control animals.

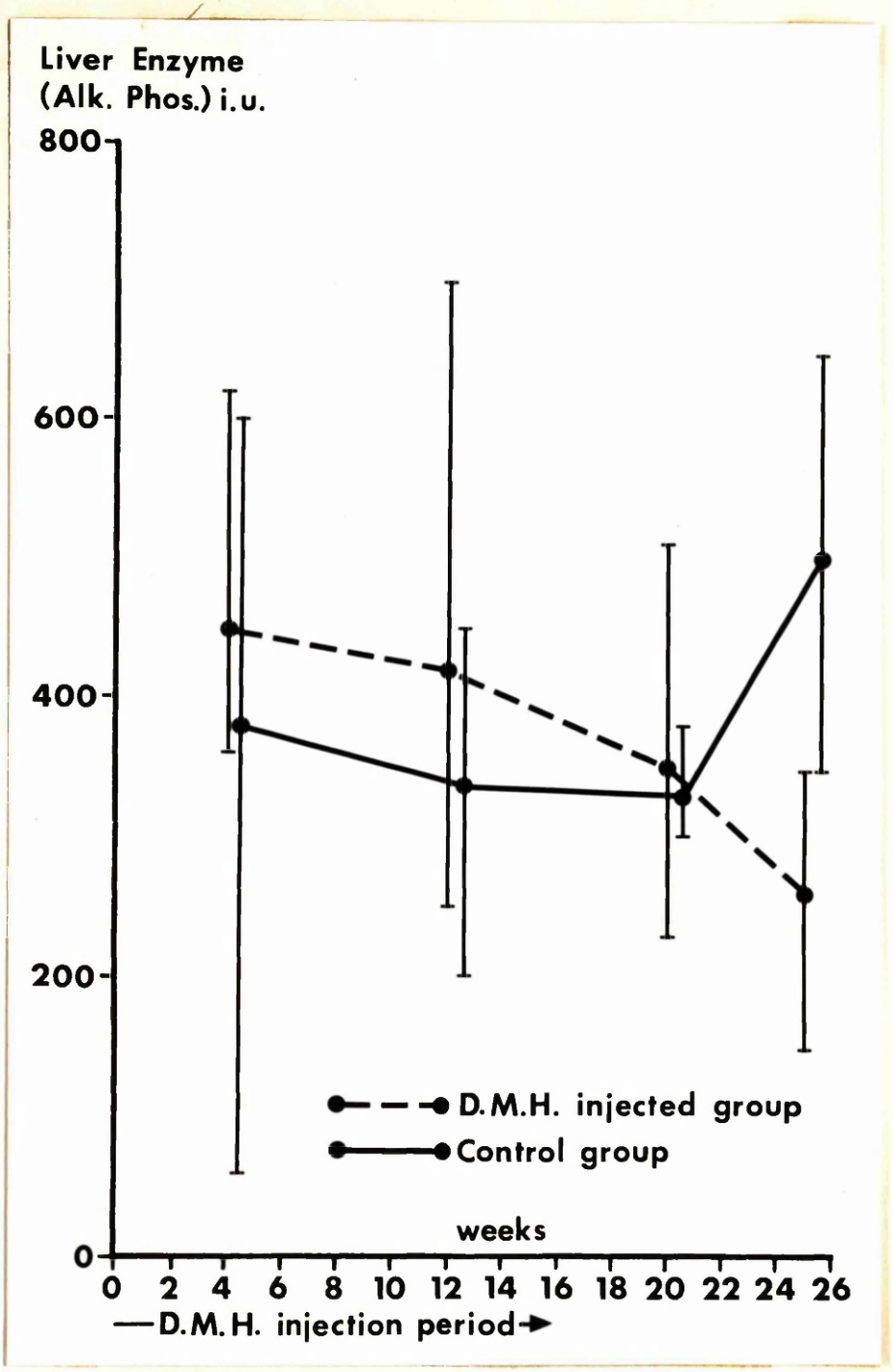


Illustration 28

Liver function tests (Alkaline Phosphatase) of the experimental and control animals

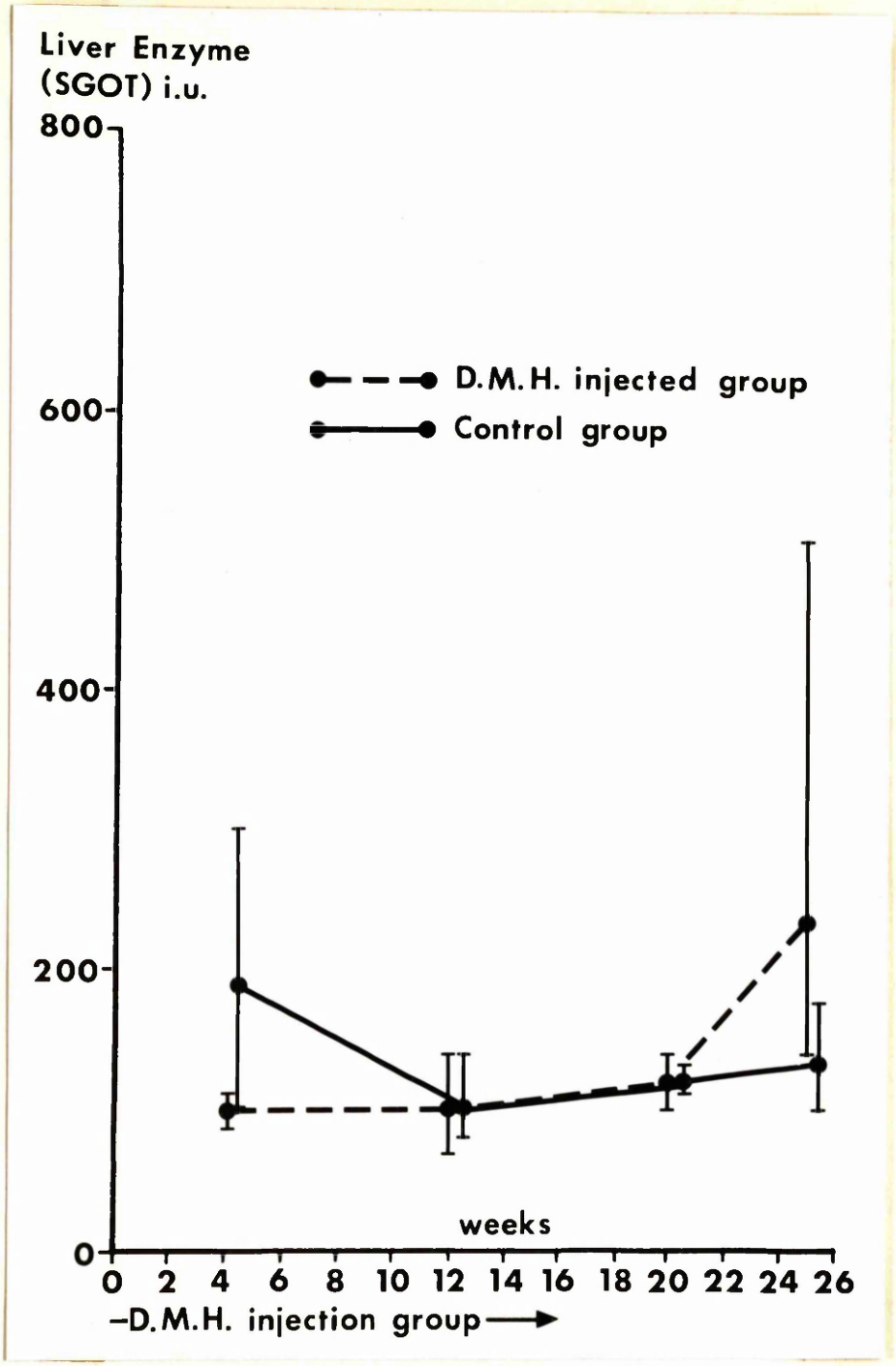


Illustration 29

Liver function tests (SGOT) of experimental animals and control animals.

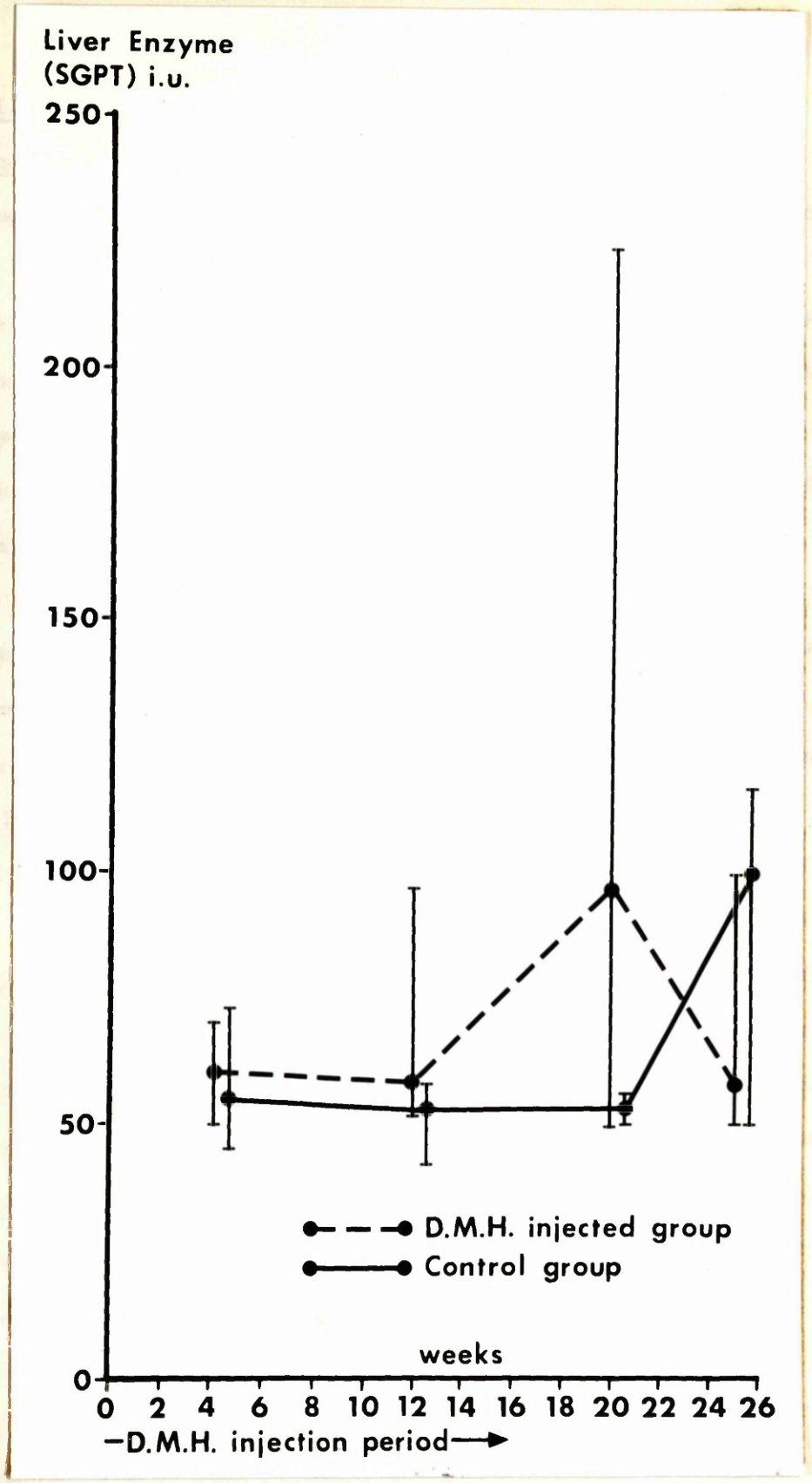


Illustration 30

Liver function tests (SGPT) of experimental animals and control animals

III. Discussion

General Condition

Roughening of the fur of the experimental animals was an obvious sign in those animals treated with Dimethylhydrazine. This became obvious after 12 weeks once the animals had received an average of 90 gm of Dimethylhydrazine. The mechanism of this change is difficult to explain other than the fact that the chemical carcinogen once it is in the circulation is toxic to any cells which are rapidly dividing in the body.

Towards the end of the experiment diarrhoea and blood in the faeces was a common phenomenon in the experimental animals. The colonic mucosa of these animals was ulcerated in areas with an extensive polymorphonuclear reaction in the tissue suggesting a colitis in some. In the majority, however, the bleeding was at the site of ulceration of a polypoidal tumour which was very often the seat of carcinomatous change. Early on in the experiment adenomas protruded into the lumen of the colon and through peristaltic action elongated and grew into polypoidal adenomas. These polypoidal tumours acted as a leading point for an intussusception. A number of these experimental animals had polypoidal tumours prolapsing from the rectum, and intussusception was the common terminal event by 30 weeks in the experiment. Metabolic collections were carried out early on in the experiment before tumours developed and were fairly accurate. Unfortunately when these metabolic collections were carried out towards the end of the experiment a number of the experimental animals had diarrhoea and blood in the stools and this made collections difficult to interpret. Another source of blood loss was from the urine which occurred in a small

proportion of the animals in the terminal phase of the experiment.

Growth

The rats were weighed weekly and all the rats had a mean weight at the beginning of the experiment of 200 grams. The weights were used as an index of the state of health of the animal. The control rats gained weight in a steady fashion over the 30 weeks of the experiment when the mean weight at that time was 650 grams, and had deposited much adipose tissue stores. The rats that were given carcinogen by 4 weeks had only put on 75% of the weight of the control rats at any period until 24 weeks when they plateaued and lost weight from then onwards until 30 weeks when they developed mechanical obstruction, and evidence of metastases. Thus, at 30 weeks the rats with colorectal cancer were lean and weighed only 55% of the weight of the controls. Their mean weight at that time was 430 grams.

The first lag in the growth curves in the rats that had colorectal cancer up to 16 weeks, was probably due to the toxic effect of the carcinogen interfering with their metabolisms, and once this stimulus was stopped they rapidly gained weight until the development of intestinal obstruction from the colonic tumours (Illustration 26).

Faecal occult blood testing was found to be positive from the twelfth week. Once tumours developed and ulceration was present, macroscopic blood was seen mixed with the excreta, and melaena was also noted on occasions. At a number of the post mortems blood was noted to be present intraluminally arising from the site of the polypoidal carcinoma, which had ulcerated. The early positive faecal occult blood

could be due to the presence of tumours but could also be related to the presence of ulceration noted to be present in the bowel of some rats which could be explained by a toxic colitis from the Dimethyl-hydrazine. The haematocrit results revealed a steady drop in the readings from 12 weeks onwards. At 24 weeks the mean haematocrit of the experimental animals was 38% (controls were 46%). These readings reflected the blood loss from the gastrointestinal tract that was obvious at this stage when it was macroscopically mixed with the faeces (TABLE VI, Illustration 27).

When faecal occult blood testing was performed early on in this animal model, it failed to show any difference between the experimental animals and the control animals. The extreme sensitivity of this test made it impossible to assess and so had to be given up very early on in the experiment.

Liver Function

None of the experimental animals ever developed signs of jaundice. The metabolism of the dimethylhydrazine involves a number of steps in the hepatocytes and therefore liver function tests were performed to assess the degree of damage and toxicity this had on the liver. Macroscopically the liver appeared to be normal and none of the animals developed liver metastases. Metastatic deposits on the liver capsule were produced in some terminal cases from transperitoneal seedlings which landed and grew on the liver surface. One experimental animal developed signs of portal hypertension with a knobbly liver. Histopathological examination of the liver in the animals revealed it to be normal apart from those animals with end stage tumours when

there was some evidence of fatty change in the liver cells and degeneration in the hepatocytes. This occurred in a setting where there was extensive tumour in the animal model (TABLE VII, Illustrations 28-30).

Attempts at inducing liver metastases by injection of aseptically minced tumour into the portal vein in a series of rats produced no liver metastases. In this experimental series the liver appeared to be relatively immune from metastatic disease which was blood borne. All the control animals receiving carrier injections were perfectly healthy with a normal histological appearance of the liver.

Renal Function

Some experimental animals had evidence of haematuria towards the end of the experiment. Histological examination of these animals' kidneys showed the presence of casts and proteinuria in the tubules. This could be explained on the grounds of some degree of toxicity caused by the Dimethylhydrazine. The renal function appeared to be satisfactory since a number of metabolic urine collections was performed on these experimental and control animals and collective urine volumes of groups of these animals did not appear to diminish in any way. In particular, no tumour metastases were found in the kidneys or primary tumours arising from the kidney.

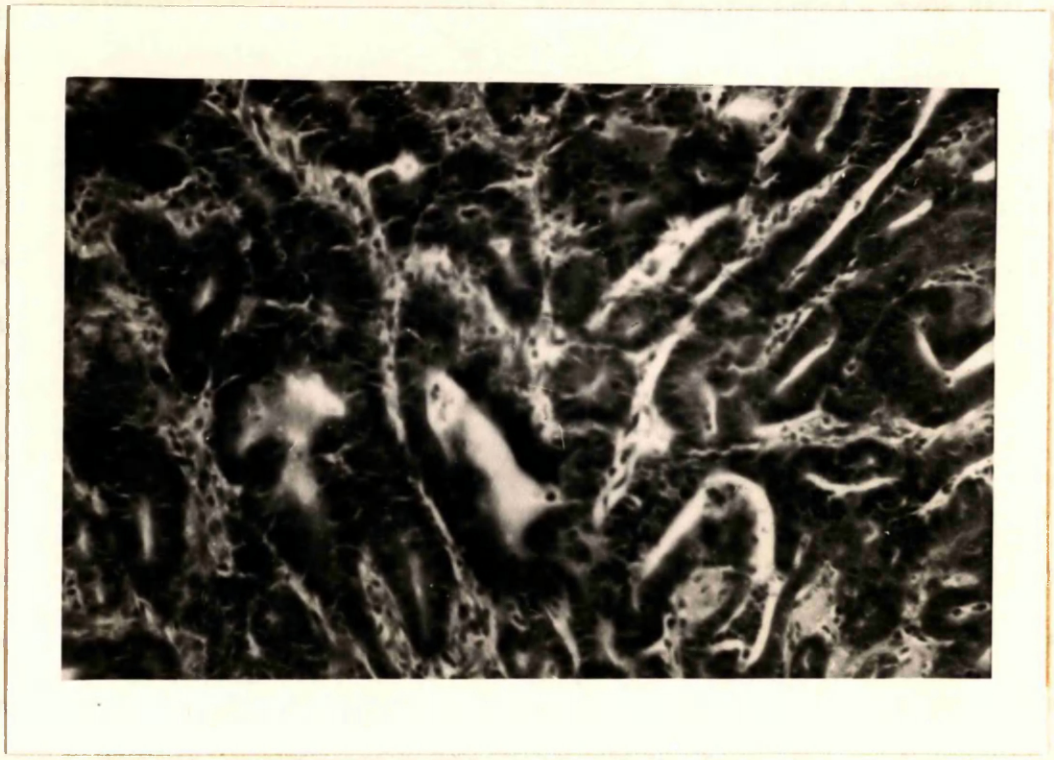


Illustration 31

A H&E Histological section of the kidney at 30 weeks after receiving DMH weekly for 16 weeks. There are tubular casts present and proteinuria. There were no glomerular changes visible.

Ear tumours

A few male and female rats, that were injected with dimethylhydrazine, developed tumours of the external ear (Weisburger 1972). This lesion was not apparent under the conditions described by other workers in B.D. strain rats (Druckrey 1970). The finding of ear duct tumours is of interest, because an entirely different type of colon carcinogen, 3-methyl-4-aminobiphenyl derivatives also induced ear duct tumours, in addition to tumours in the colon (Walpole 1960). Rats injected with azoxymethane for 12 weeks and sacrificed 12 weeks later exhibited a high incidence of ear duct tumours.

In the existing experimental model, ear tumours appeared in some rats treated with dimethylhydrazine. These tumours appeared as little lumps initially on the posterior aspect of the ear which then grew and became obvious at about 28 weeks in the experiment. Some ulcerated and exuded a cheesy sebaceous material. The histopathology of these ear tumours showed them to be squamous cell in origin, and arising from the middle ear. The middle ear must be another target organ for the carcinogen used in this experimental animal (Illustration 32).

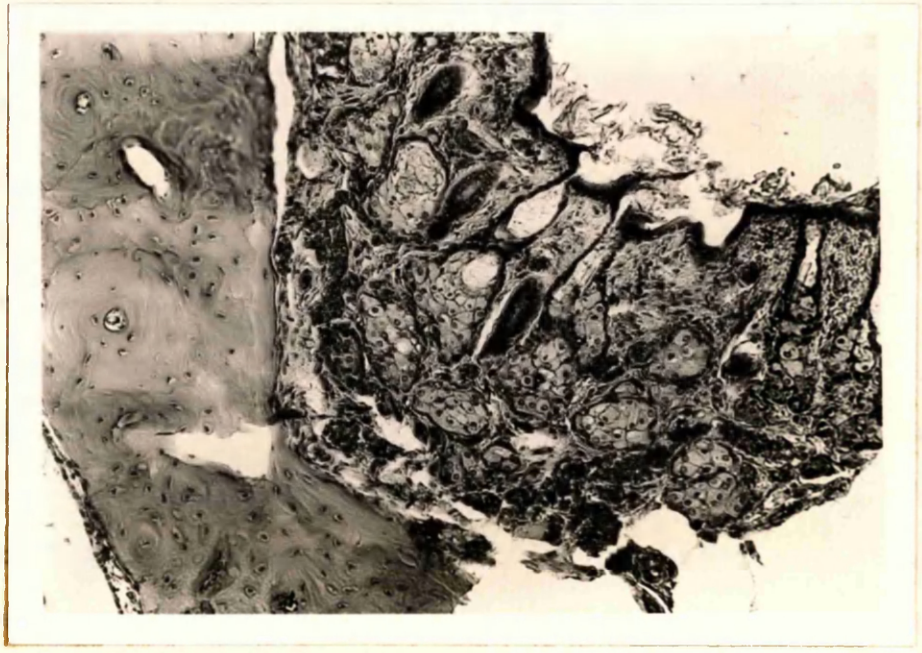


Illustration 32

Histological section of a squamous cell tumour arising from the middle ear, which appears to be another target organ for DMH. These tumours appeared terminally in a few rats who had tumours induced in their colon by DMH. These tumours were adenocarcinomata and distinct from the ear tumour.

This was the only other target organ to be found sporadically involved after a full post mortem examination of all the viscera was performed. The occurrence was not dose related or diet related in any way.

CHAPTER FIVE. FACTORS INVOLVED IN THE AETIOLOGY OF TUMOUR INDUCTION.

Design of the experiments to test the hypothesis that alteration of these factors may alter the natural history of this experimental model for colonic dysplasia.

Colonic dysplasia induced by Dimethylhydrazine (chapter three) is the precursor of the colonic polyps and colonic neoplasia found in the experimental model described by Martin et al 1973 (chapter two). On withdrawal of the carcinogenic stimulus used in this model, these areas of dysplasia could be affected by factors acting locally which are present in the colonic environment. These factors include the type of diet ingested, the faecal bile acids which are known tumour promotors, the faecal residue, the lack of which has been incriminated in the high incidence of colonic neoplasia in western diets, and the intestinal transit time. The longer it takes toxic substances to be in contact with the areas of colonic dysplasia, the more these lesions are predisposed to develop into neoplasia. The microflora of the colon is determined by the diet and the faecal bile acids, and enters into a complex relationship as an added factor in the colonic environment. Finally the presence of environmental carcinogens present in food stuffs could act as a continuous stimulus acting both as an initial inducer of the colonic dysplasia as well as a promotor for neoplasia.

Dietary Fibre

The term "dietary fibre" is the plant material that is resistant to hydrolysis by the digestive enzymes of man. Although it provides only

100 calories per 25 grams, it is composed of a group of compounds with physical, chemical and metabolic properties. Dietary plant fibre consists of cellulose, pectic substances, hemicellulases, polysaccharides and lignins (TABLE VIII).

Cellulases are moderate water absorbers, whereas polysaccharides which are variable esterified acidic sugar polymers are strong absorbers of water, and act as weak ion exchange resins.

The mechanism of action of dietary fibre in the gastrointestinal tract can be described as having three effects. The first effect is the bulk effect which is produced by the hygroscopic properties of cellulose in the dietary fibre. A second effect is the osmotic effect produced by the short chain fatty acids. The third effect is the cathartic effect produced by sequestration of the bile salts by the dietary fibre and their eventual release in the colon.

Lignin consists of hydroxylated aromatic polymers which are also absorbers of acidic compounds. Water added to some Polysaccharides causes them to swell and form a gel. Substances such as carageenes, cellulases and the polysaccharides found in wheat bran have a large capacity to hold water. Acidic polysaccharides, alginic acid and hemicullulases may also bind cations. The cation exchange capacity is therefore dependent on the plant fibre in the dietary fibre eaten.

The effects of dietary fibre have been reported to increase the faecal weight, speed up the intestinal transit time, alter the bacterial flora and alter the organic anions in the colonic lumen which include

bile salts and short chain fatty acids (Burkitt 1971).

Dietary fibre is essential for normal bowel function, promoting regularity, soft stools and a rapid transit time. One of the most obvious differences in the diet of western world countries compared to other less well developed countries is the ratio of unabsorbable fibre and refined carbohydrate.

Dietary fibre metabolism involves breakdown of the fibre by colonic bacteria cellulases which produce short chain fatty acids. These fatty acids include acetic acid, butyric acid and proprionic acid. There is also production of methane, carbon dioxide and water.

Epidemiological surveys confirmed the marked variation in the geographic incidence of colonic cancer. The dietary habits and environmental carcinogens have been implicated in the aetiology of this cancer (Burkitt 1972). It was found that for cancer of the colon, the highest correlation is to dietary animal fat. (Armstrong and Doll 1975). Other workers relate the risk of large bowel cancer to the use of refined sugar and dietary deficiency of fibre (Burkitt 1971). A review of populations in 37 countries revealed no correlation between colonic cancer and dietary fibre (Drasar and Irving 1973). These workers although not finding a correlation with the fibre content did find a correlation with the amount of refined sugar taken in the diet. Other workers established a positive correlation between colon cancer and the intake of legumes, which in this instance happens to have a high fibre content (Haenszel 1973). In spite of all this contradictory evidence, high risk populations do exist and their diet has been found

to contain large amounts of animal fat and protein. Migrant studies have shown that these groups who move from a low risk area to a high risk area assume the risk of the geographic area they are in (Berg et al 1973). From the epidemiological evidence, an environmental factor which is probably in the diet plays an important role in the aetiology of colon cancer. Any attempt to test these hypotheses requires a reliable animal model in which strict controlled studies can be performed (Illustration 33).

More recent epidemiologic studies have identified two dietary factors, a relatively high intake of fat and a relatively low intake of fibre, that are associated with colon cancer in humans. (Reddy 1978)

However, a recent study has shown a low risk of large bowel cancer in a rural Finnish population with a high dietary intake of fat, but also a high intake of fibre. Observations in humans and studies in animals have indicated that dietary fibre may protect against colon carcinogenesis by binding bile acids in the intestinal tract, by a direct effect on the colonic mucosa and by an indirect effect on the metabolism of carcinogens. The strength of protection varies with the type of fibre. (Reddy 1980)

Main Group	Class	Units
Carbohydrates	Cellulose	Glucose
	Hemicellulose A	Xylose
		Galactose
		Glucose
		Mannose
		Arabinose
	Hemicellulose B	Acid Hemicelluloses (uronic acids)
	Pectic substances	Galacturonic acid
Non-carbohydrate	Lignin	Phenyl-propane

TABLE VIII

COMPONENTS OF DIETARY FIBRE

(Mixture of high molecular weight polymers)

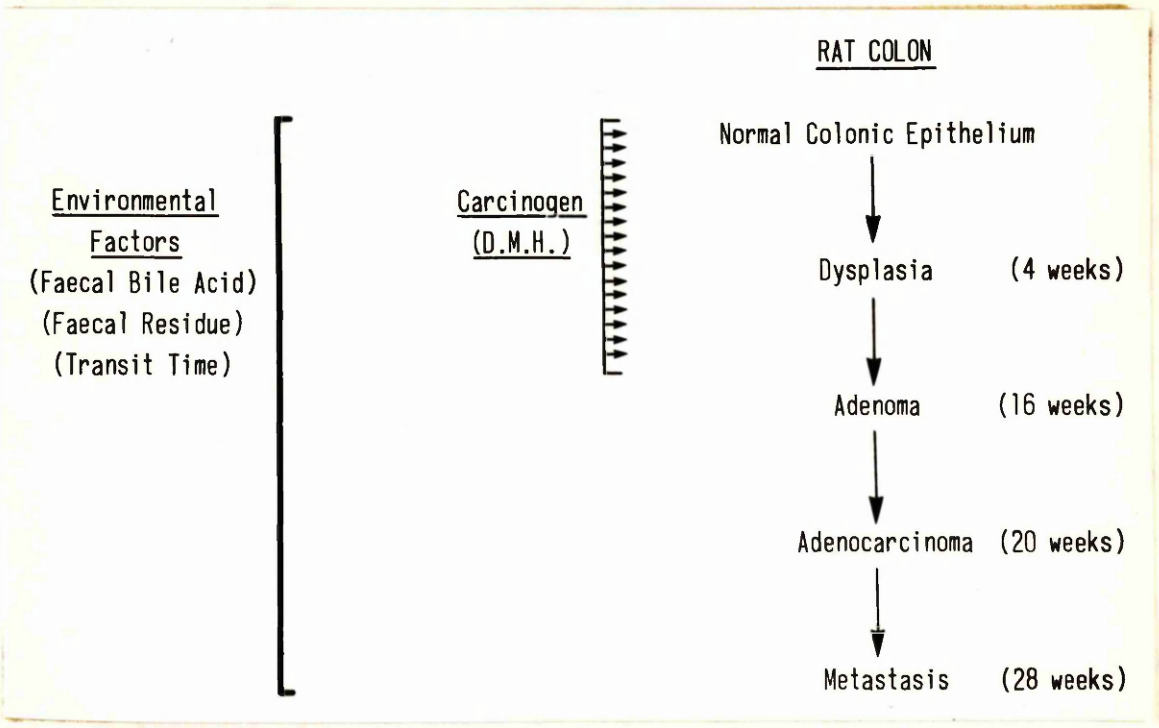


Illustration 33

Environmental factors influencing the development of tumours induced by DMH.

Design of experiments to test the hypothesis, illustrated in diagram.

The first set of experiments using the model for colonic dysplasia is set up to test the effect of dietary fibre on the natural history of the tumours induced in the model. The experimental animals receive a high fibre isocaloric diet in an attempt to alter the colonic environment and so alter the promoting factors which could be acting on the areas of dysplasia in the colon. The experiment is terminated at 28 weeks and a comparison made between the neoplasia induced in those rats on a high fibre diet and those on a low fibre diet. In particular the total number of neoplasia, their anatomical location in the colon, as well as their degree of differentiation and invasion of tissues is studied.

If the above experiment showed any difference between the groups on a high fibre diet compared to the groups on a low fibre diet, then the next set of three experiments is carried out using the metabolic cages described. (CHAPTER ONE). These experiments involve the use of three groups of rats with ten rats in each group. One group would serve as controls, and the other two groups would receive carcinogen to induce colonic dysplasia, one group fed on a high fibre diet as in the first set of experiments while the other group are fed on a low fibre diet.

The first of these experiments is set up to measure the faecal bile acids at different time points in the experiment in each rat of the different groups.

The second of these experiments is set up to measure the intestinal transit time in each of the three groups of rats before tumours develop, at 12 weeks, and again at 26 weeks after tumours have developed.

The third factor measured in the last of these experiments is the faecal residue in each of the three groups. The weight of the faecal pellets as well as the faecal volume as an index of the faecal bulk measured from 24 hour collections of the groups of rats in metabolic cages.

Finally in the last of these experiments, the experimental animals are sacrificed to determine whether the interference and invasiveness of the techniques used in measuring these factors had in any way altered the development of colonic neoplasia from the colonic dysplasia induced by the carcinogen.

CHAPTER SIX. DIETARY FIBRE

Epidemiological surveys have shown a striking variation in the geographic incidence of colonic neoplasms (Burkitt 1972). A number of workers have implicated exogenous carcinogens in the environment to explain this variation in incidence (Burdette 1974). Others like Burkitt have brought to the forefront the highly controversial issue relating the effect of dietary fibre to colonic carcinogenesis (Burkitt 1972). In an attempt to study this problem, the experimental model for inducing colonic dysplasia by using Dimethylhydrazine was used. The effect of changes in the dietary fibre composition on the induction of these tumours and their rate of growth is investigated.

I. Materials and methods

(a) Animals

Groups

Group A: Twenty rats on a high fibre diet were injected subcutaneously with DMH on a weekly basis for a period of 16 weeks. Their diet consisted of Oxoid 41 without antibiotics, with non-processed bran (15% crude fibre and 1.5% digestive fibre) added to the diet.

Group B: Twenty rats on a normal diet which had a low fibre content (crude fibre 2.5% and digestible fibre 1.5%). These rats were injected with carcinogen as the rats in Group A.

Group C: Twenty rats on a normal diet (crude fibre 2.5% and digestible fibre 1.5%) received carrier substance (EDTA) by injection only.

(b) Operative procedure and measurements

Prior to the experiments the rats were allowed a week to settle in their cages and sample the various diets. In order to ensure the quantity of the food presented was eaten, the cages were inspected for residue and recorded if found. The caloric value of the diets was made to 11.2 megajoules per kilogram of diet, so that they were all isocaloric. The rats were weighed weekly and this was used to assess the health and well-being of the rats prior to their weekly injections.

Five rats from each group were sacrificed at four time points in the experiment: at 20 weeks, 23 weeks, 26 weeks and 30 weeks. The colon was opened and pinned on to a cork board to facilitate examination. The number and anatomical location of the tumours was noted as well as whether they were plaques, sessile or polypoidal in shape. The tumours were individually dissected off the colon and weighed using a microbalance. The results were expressed as a ratio of the total body weight of the animal. Histological examination of all the tumours was performed and regional lymph nodes were examined for evidence of tumour metastases. The major organs were also examined for signs of tumour spread and evidence of toxicity from the carcinogen.

(c) Recordings

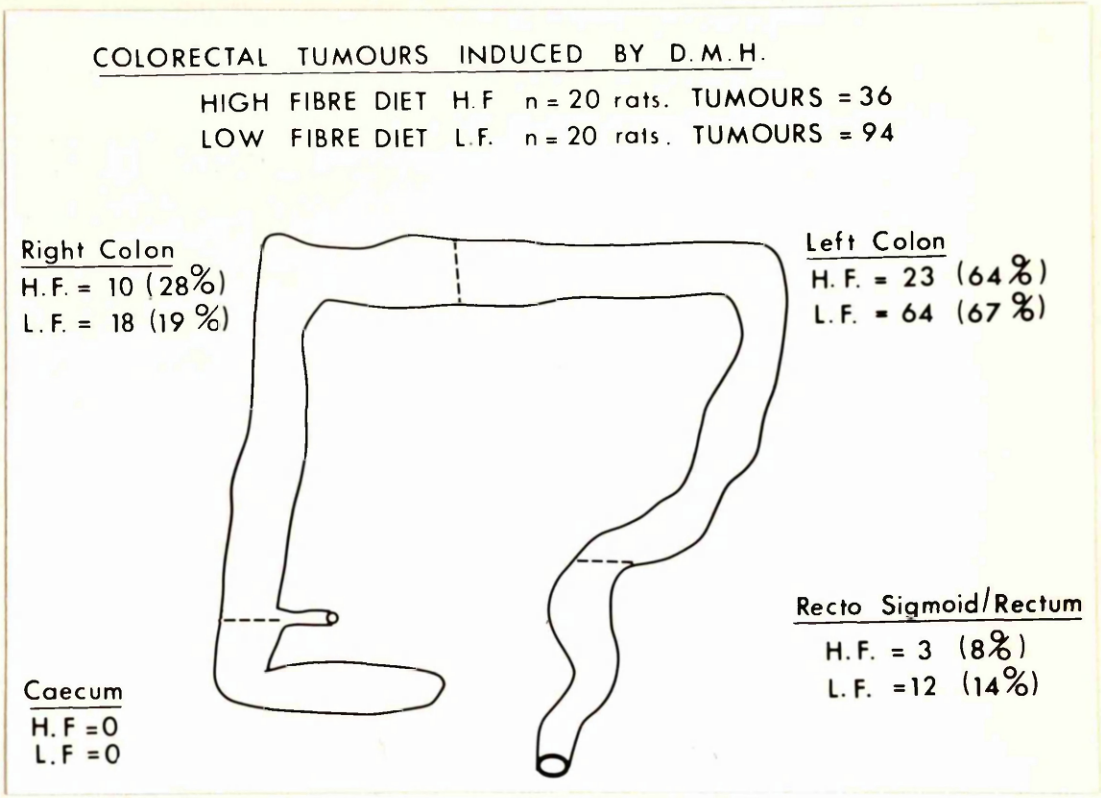


Illustration 34

The anatomical distribution of the colorectal tumours induced in the two groups of animals. The percentage of the total tumours induced in the group is given in brackets next to the actual numbers induced. The tumours are predominantly left sided with a significantly greater number of neoplasia induced in the rats on a low fibre diet. No tumours were found in the caecum in either group.

HISTOPATHOLOGICAL DIFFERENTIATION

	High Fibre Diet	Low Fibre
Plaque  Dysplastic lesions	13	17
Sessile  Adenomatous lesions	12	44
Polypoidal  Adenocarcinoma	11	33
Hepatic metastases	0	2
No. Rats	20	20

TABLE IX

Analysis of the tumours induced showed a slight preponderance of dysplastic lesions in the low fibre group which was not statistically significant. There were four times as many adenomatous lesions in the rats on a low fibre diet and three times as many polypoidal adenocarcinomata in the rats in this group. In addition there were metastases in the low fibre group rats, which were not present in the high fibre group. The two rats that developed hepatic metastases were rats in the low fibre diet group.

II. Results

All the control animals receiving the carrier injections (EDTA) were perfectly healthy with a normal histological appearance of colon, liver and kidneys. Failure to thrive and weight loss appeared as early as 12 weeks in some rats. Other signs of morbidity included roughening of the fur, diarrhoea, blood in the faeces, prolapsing rectum and terminal obstruction of the intestine with ascites.

Body Weight

Control rats progressively gained weight over the 30 weeks but rats injected with DMH did not put on as much weight during the 16 weeks of injections. Once the injections were stopped they gained weight until the development of intestinal obstruction due to the colonic tumour. They never caught up with the control rats but there was no significant difference in weight. The weights became less meaningful from 20 weeks onwards when tumours appeared and caused mechanical effects on food ingestion. Thereafter weights of the experimental rats in Groups A and B did not vary except terminally when they lost weight from inanition if they developed tumours.

Anatomical Location

A total of 36 tumours were induced in the rats on a high fibre diet compared to 94 tumours in the rats on a low fibre diet. The anatomical location showed them to be predominantly left-sided in both groups. No tumours were found in the caecum (Illustration 34).

Ten tumours were found on the right side (28%) in rats on a high fibre diet, compared to 18 (19%) on a low fibre diet. Twenty-three were left

sided (64%) in rats on a high fibre diet compared to 64 (67%) on a low fibre diet. Three tumours (8%) were found in the rectum and recto-sigmoid in the high fibre group and 12 tumours (14%) were found in the low fibre group. There was no statistical difference between rats on a high fibre and a low fibre diet in the anatomical site of the tumours induced.

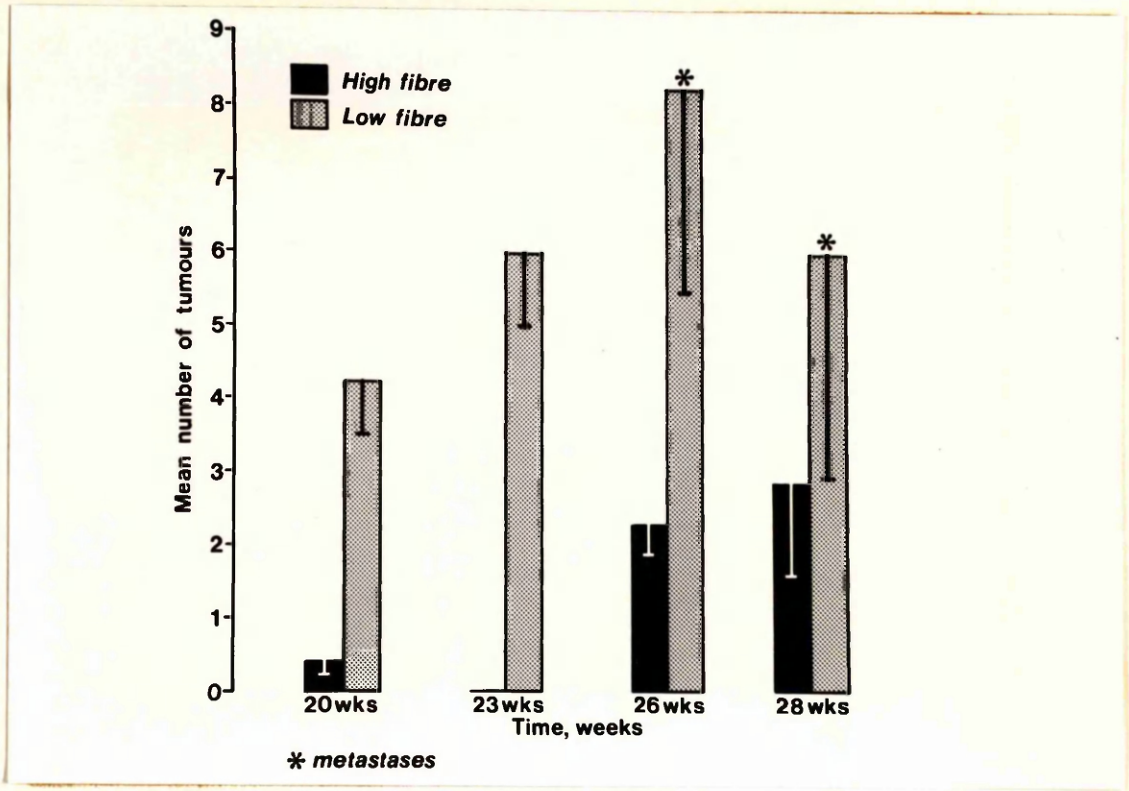


Illustration 35

Mean number of tumours induced at four time intervals in the experiment, demonstrating the greater number of tumours induced in the rats on a low fibre diet and the presence of metastases in this group at 26 weeks and at 28 weeks.

The mean number of tumours produced in the experimental rats was consistent at the four time intervals. The number of colorectal tumours induced in each rat ranged between 6 and 8, and many of the tumours were found in clusters in the rats on a low fibre diet (Illustration 35).

Histopathology

The tumours were classified into plaques which showed only dysplastic changes, sessile adenomas histologically ranged from simple adenomas to carcinoma in situ, and polypoidal carcinomas which showed invasion of the stalk often the cause of intussusception and haemorrhage terminally. The rats on the high fibre diet had a similar number of dysplastic lesions (plaques) as the rats on a normal fibre diet. Only 12 adenomatous lesions were found in rats on a high fibre diet compared to 44 in those on a normal diet. Eleven polypoidal adenocarcinomas were found in the high diet compared to 33 found in the normal diet group (TABLE IX).

Rats on a high fibre diet developed fewer colonic tumours which were better differentiated and less invasive. Only 2 rats in the whole group developed multiple liver metastases and these were in the normal diet group (Illustration 39). The commonest tumour found was a moderately well differentiated adenocarcinoma.

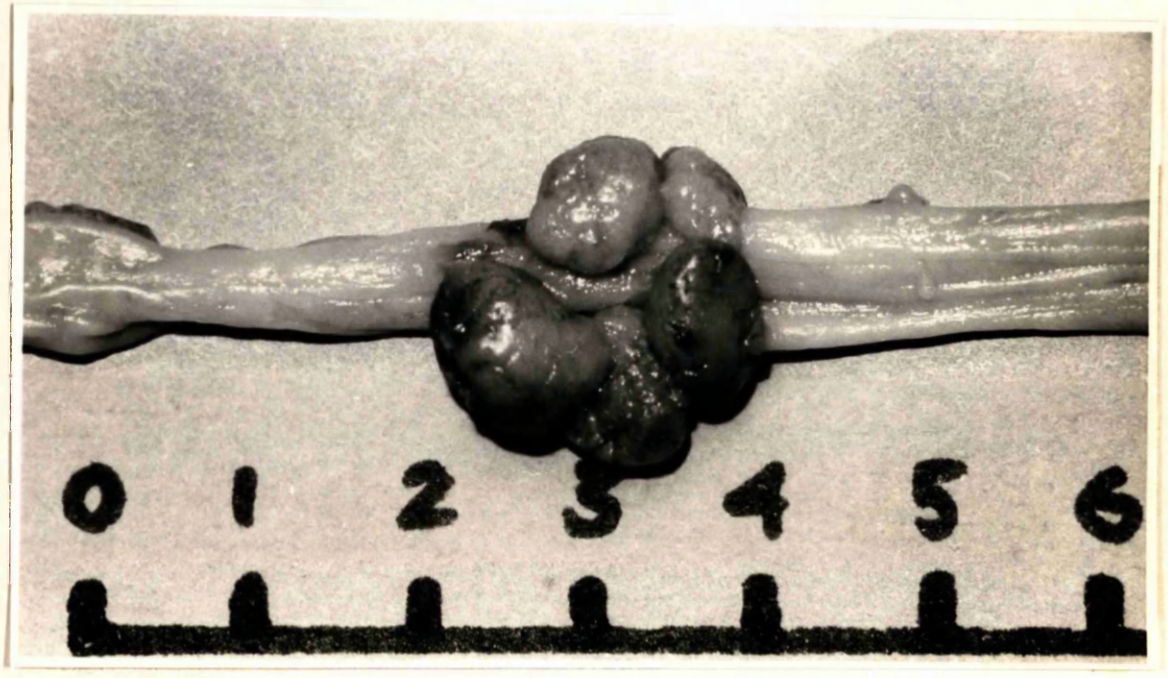


Illustration 36

Multiple synchronous polypoidal tumours were induced in the rat colon: many arose as clusters at the sites where the epithelium had become unstable by DMH injections for 16 weeks. These tumours grew from adenomata and the surfaces eventually became carcinoma in situ which went on to invade the stalk of the polyp and metastasise through this route.

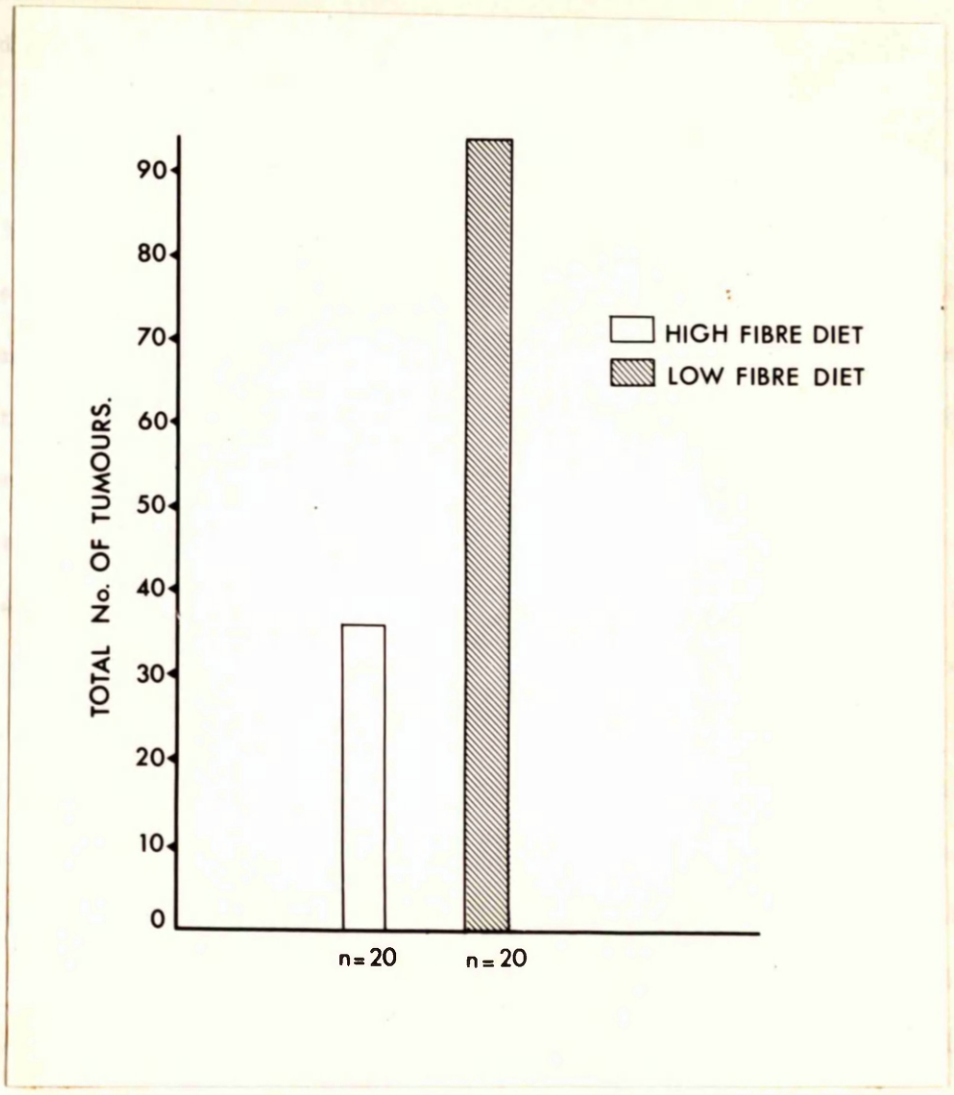


Illustration 37

The total number of tumours induced in rats on a high fibre diet and rats on a low fibre diet is illustated in this histogram. A total of 36 tumours were induced in the rats on a high fibre diet compared to a total of 94 tumours that were induced in the rats on a low fibre diet. Both groups received DMH for 16 weeks at the beginning of the experiment and the animals were sacrificed at 28 weeks into the experiment.

Tumour Growth

After dissecting out all the colonic tumour tissue the total bulk of tumour was weighed on a microbalance. The relative growth of the tumour was expressed as a ratio of the colonic tumour mass to the total body weight. Illustration 38 demonstrates the main number of tumours induced in each group of rats at four time points in the experiment. Each time point represents 10 rats. The results were statistically significant at 20 weeks, 23 weeks and 26 weeks. This represents the growth of tumour bulk in relation to the body weight of the animal. At 20 weeks the ratio was 0.004% in the high fibre group compared to 0.04% in the low fibre group. At 23 weeks the percentage in the high fibre group was less than 0.004% while the low fibre group was 0.15%. By 26 weeks the high fibre group was 0.05% compared to 0.27%. At 30 weeks the ratio in the high fibre diet was 0.13% while the low fibre diet was 0.11%. This was because a considerable amount of extra- colonic tumour from metastatic disease in those rats on a normal fibre diet was not measured and produced a falsely low reading. The only 2 rats in the whole experiment that developed liver metastases were rats on a normal fibre diet. The mean weight of the rats was 450 grams (standard deviation $\pm$ 0.2). At 30 weeks extra-colonic tumour metastases were present in a number of the rats on a normal fibre diet and therefore the ratio was much lower since many of these metastases could not be weighed individually (Illustration 38).

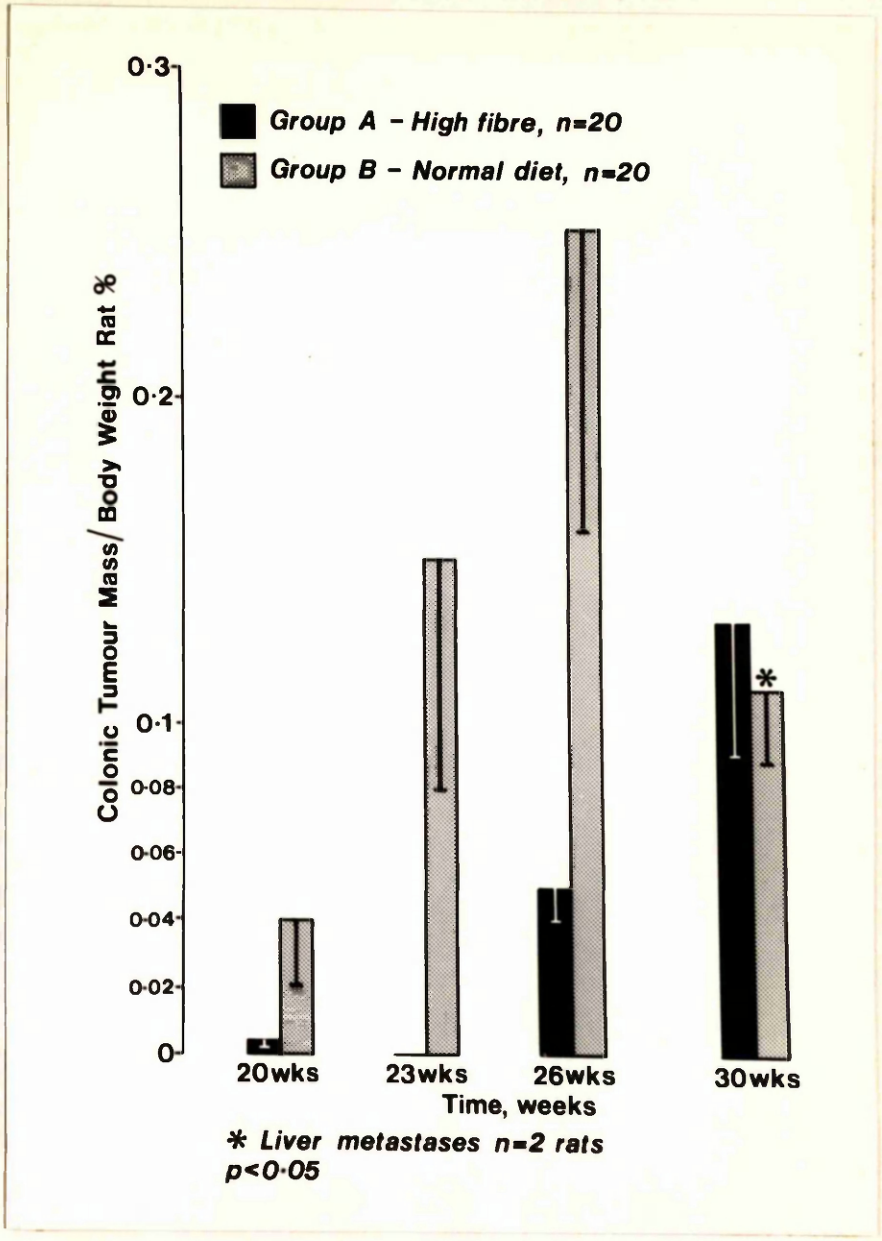


Illustration 38

Histiogram of the ratio of colonic tumour mass and the body weight as an expression of tumour growth. There was a significant difference between the two groups at 20 weeks, 23 weeks and 26 weeks into the experiment. At 30 weeks there was no significant difference between the groups because extra colonic tumour was not accounted for as many of the animals in Group B had evidence of metastatic disease and some died from it.

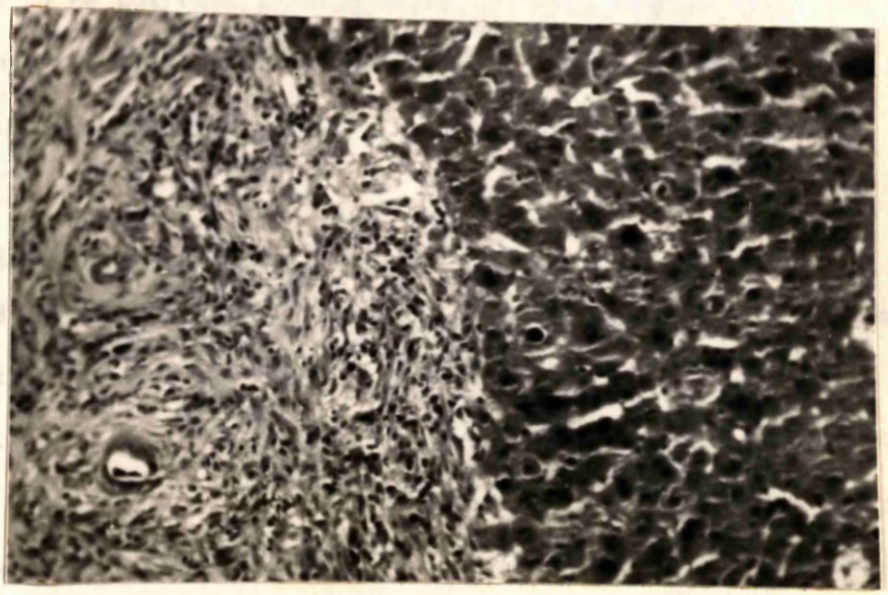


Illustration 39

Histology of a metastatic deposit of an anaplastic carcinoma arising from the colonic polypoidal neoplasm in a rat that received DMH and was on a low fibre diet. The histology of the metastatic lesion was similar to that of the primary tumour, showing a poorly differentiated anaplastic carcinoma, seen on the left of the photograph, and normal liver architecture on the right hand side.

Other Features

No tumours were found at the injection sites. At 30 weeks the liver showed some evidence of fatty and other non-specific degenerative changes. All the tumours encountered were carcinomas and no sarcomas were seen in these rats. All the control animals receiving the carrier injections were perfectly healthy with a normal histological appearance of the colon and liver and kidneys. Faecal occult blood testing was found to be positive from 12 weeks and once tumours developed with ulceration, there was altered blood mixed with the excreta. The site of bleeding was areas of ulceration in the polypoidal carcinoma.

Statistics

At 20, 23 and 26 weeks, rats on a high fibre diet had a significantly lower ratio of tumour to body weight than rats on a normal fibre diet ($P<0.05$). Non-parametric statistical test shows significance at 20 and 23 weeks when the student T test did not. (Mann-Whitney rank sum test) At 30 weeks no significant differences were shown between the two groups because the metastases were not accounted for in rats on a normal fibre diet.

Week 20

		No. of Tumours	
L.F.			
x = 0.0402	0.11204	4	x = 4.25
SD = 0.0479	0.013	5	SD = 1.71
sen = 0.0240	0.017	2	sen = 0.85
(n = 4)	0.01893	6	(n = 4)
H.F.			
	0.00621		
	0.00958	1	x = 0.40
x = 0.0032		1	SD = 0.55
SD = 0.0045		0	sen = 0.24
sen = 0.0020	(n = 5)	0	(n = 5)

Non-parametric statistical test showed significance at 20 weeks.
(Mann-Whitney rank sum test)

Week 23

		No. of Tumours	
L.F.			
x = 0.1585	0.33767	9	
SD = 0.1731	0.0151	4	x = 6.17
sen = 0.0707	0.03167	7	SD = 3.13
(n = 6)	0.01276	1	sen = 1.28
	0.1509	7	(n = 6)
	0.40303	9	
H.F.			
x = 0		0	0
SD = 0		0	0
sen = 0	0		0
(n = 2)			(n = 2)

Non-parametric statistical test showed significance at 23 weeks.
(Mann-Whitney rank sum test)

Week 26

		No. of Tumours	
L.F.			
$\bar{x}$ = 0.2718	0.04312	2	$\bar{x}$ = 8.33
SD = 0.2022	0.3458	11	SD = 5.51
sem = 0.1167	0.42662	12	sem = 3.18
(n = 3)			(n = 3)
H.F.			
$\bar{x}$ = 0.0509	0.02916	1	$\bar{x}$ = 2.33
SD = 0.0390	0.10864	2	SD = 1.22
sem = 0.0130	0.05546	4	sem = 0.41
			(n = 9)
(n = 9)	0.04368	3	
	0.02157	2 (1pl)	
		0	
	0.11865	3	
	0.03875	3	
	0.0418	3	

L.F.XH.F. t = 2.8377 0.01<P<0.02, significant.

Week 30

		No. of Tumours	
L.F.			
$\bar{x}$ = 0.1105	0.13368*mets	2	$\bar{x}$ = 4.00
SD = 0.0328	0.8727 *mets	6	SD = 2.83
sem = 0.0232			sem = 2.00
(n = 2)			(n = 2)
H.F.			
$\bar{x}$ = 0.1371	0.17867	5	$\bar{x}$ = 4.00
SD = 0.0589	0.09544	3	SD = 1.41
sem = 0.0416			sem - 1.00
(n = 2)			(n = 2)

L.F.XH.F. 0.3966 0.7<P<0.80, not significant

III. Discussion

Dietary fibre is the plant material that is resistant to hydrolysis by the digestive enzymes of man (Trowell 1972). Although it provides only 100 calories per 25 grams, it is composed of a group of compounds with physical, chemical and metabolic properties. (Kritchevsky 1983*)

Some workers have reported that dietary fibre is essential for normal bowel function, promoting regularity, soft stools and a rapid transit time. One of the most obvious differences in the diet of western world countries compared to other less well developed countries is the ratio of unabsorbable fibre to refined carbohydrates (Burkitt 1971). Epidemiological surveys confirm the marked variation in the geographic incidence of colonic cancer and dietary habits and environmental carcinogens have been implicated in the aetiology (Burkitt et al 1972). Epidemiologists found that for cancer of the colon the highest correlation is to dietary fat, especially animal fat (Armstrong and Doll 1975). The risk of large bowel cancer has been related to the use of refined sugar and dietary deficiency of fibre (Burkitt 1975). Other authors show no correlation between the dietary habits and the development of colon cancer. In a review of 37 countries, no correlation was found with the dietary fibre habit of these populations. These workers found no correlation with the fibre content but found a correlation with the amount of refined sugar that was taken in the diet of these people (Drasar 1973). Yet another worker established a positive correlation between colon cancer and the intake of legumes which in this instance happens to be high in fibre content (Haenszel 1973). In spite of all this contradictory evidence, high risk populations do exist and their diet has been found to contain large

amounts of animal fat and protein. Migrant studies have shown that these groups moving from a low risk area to a high risk area assumed the risk of the geographic area they are in (Berg 1973). Therefore from the epidemiological evidence an environmental factor which is probably in the diet plays an important role in the aetiology of colon cancer (Weisburger 1982*). Any attempt to test these hypotheses requires a reliable animal model in which strict controlled studies can be performed.

The incidence of spontaneous colonic cancer in rats has been reported at 0.1% (Miwa 1976). The discovery of the carcinogenic effect of cycasine and its aglycone on intestinal mucosa by Laqueur opened the way for the development of new synthetic intestinal carcinogens (Laqueur 1965). Druckrey demonstrated that the synthetic dialkyl-hydrazines were organotropic for gastrointestinal mucosa (Druckrey 1967).

The mode of administration is important since a single injection of methylazoxymethanol produces colonic polyps as well as small bowel tumours (Reuber 1976) whereas an intrarectal infusion of the same carcinogen produced squamous cell carcinomas and myosarcoma (Narisawa 1971). Administration of dimethylhydrazine in a dose of 15 mg/kg body weight for 7 months produced both colonic tumours and small intestinal tumours in 60% of rats with hepatotoxicity and a liver fibrosarcoma (Martin et al 1973). When a total lower dose of methylazoxymethanol was administered intraperitoneally, male rats developed multiple polypoidal carcinomas in the left colon (Reuber 1976). In the model used for these experiments a low dose of carcinogen was used which was sufficient to produce a set number of dysplastic areas in the colon in

each rat which spontaneously underwent changes progressing to adenomatous polyps with subsequent production of carcinoma. Similarly few toxic side effects from the carcinogen were seen causing minimum interference with the rate of tumour growth in the animal. This model is reliable and easily reproducible with a high percentage of tumour induction in a relatively short period of 18 to 20 weeks (Carachi et al 1979). Recent work has demonstrated the superiority of this model and the relative ease of tumour induction (Martin 1982*)

This model was used to study the effect of altering the colonic environment by using isocaloric high fibre diet in male Sprague-Dawley rats with a set number of colonic dysplastic lesions induced by a low dose of DMH. Some authors have shown no difference in their experimental groups, the carcinogen dose used was 40 mg/kg of DMH which increased the malignant potential of the target cells (Cruse et al 1978). Other authors who found no difference between their groups said that the high tumour frequency in their model made the demonstration of the weak protective effect of wheat bran difficult to detect (Bauer et al 1978).

After subcutaneous injection, 1,2 dimethylhydrazine hydrochloride enters the blood stream and is then metabolised by the endoplasmic recticulum in the hepatocytes. Hydroxylation in the liver, changes the compound into bioactive amines, azomethane and azoxymethane. These compounds are then conjugated and excreted as beta-glucuronide in the bile. The enzyme beta-glucuronidase in the colonic bacterial flora breaks down the metabolites and converts this compound to methyl-azoxymethanol (Weisburger 1971). In germ-free animals this does not occur, most of the dimethylhydrazine can be collected in the urine and

faeces and no tumours develop (Wiebecke 1969). Spontaneous breakdown to methyldiazonium then occurs and the methonium ion is believed to be the final alkylating agent for carcinogenesis. The colonic mucosal environment is determined by the conditions prevailing intraluminally. Experimental evidence in animals with colostomies shows that they developed fewer tumours in the defunctioned bowel than did intact animals in the same bowel segment (Campbell et al 1975). The faecal stream alters the carcinogenic activity of azoxymethane but it can also reach its target tissue by a route other than the faecal stream which is likely to be through the blood stream (Druckrey 1972, Preussmann et al 1972). The preponderance of left-sided lesions in rats on the normal low fibre diet in this experiment can be explained by the increased concentration and duration of contact by the carcinogen on the colonic mucosa. The protective role of the fibre in the diet may be due to a number of factors acting directly or indirectly affecting the faecal bulk, the transit time, alteration of colonic flora, faecal bile acid production and possibly the absorption of carcinogenic ions by the dietary fibre deactivating it (Cummings 1978*).

The incidence, site of tumour induction, histological features and morphological behaviour make it an ideal model for studying colonic environmental factors which may protect the target cells from the environmental carcinogens. Chemical carcinogens in the environment could well play a role in the aetiology of colon cancer since as much as 2,500 chemical substances are routinely added to our daily food-stuffs in western society (Kermode 1972). The final breakdown products of DMH metabolism are the same as those of dimethylnitrosamine. Nitrites are also common agents used to preserve meat and fish

products and amines are widely distributed in a number of foods and drugs. (Lowenfels and Anderson 1977). All of the ingredients necessary for the production of a potent carcinogen are available in a western diet and nitrosamines must be strongly considered in the genesis of cancer. A number of microsomal oxidative systems have been identified which help to detoxify a variety of carcinogens. The activity of these systems have been shown to be increased by the ingestion of a number of vegetables and fibres. Similarly a number of plant fibres and fibre-containing materials had marked activity in counteracting toxic effects of chemicals induced in rats (Lijinsky and Epstein 1970).

More recent experimental animal studies suggest that fibre in the diet alters the promotional events in the colon tumorigenesis rather than affecting the initiation reaction between carcinogen and cell. Fibre containing diets can prevent carcinogen from being absorbed in the intestine and some component of bile acid metabolism may play a role in promoting colon tumour development (Zedeck 1980*. Glauert 1983*).

In this experimental model for multiple polyps dietary fibre appears to be a factor which directly alters the colonic environment. It protects the colonic mucosa from the alkylating carcinogen DMH, retards the growth rate of colonic polyps and their potential for malignant change. The tumours induced in rats on a high fibre diet were morphologically less aggressive, and better differentiated on histological grading. Multiple hepatic metastases from an anaplastic adenocarcinoma were found in the rats that were not protected by a high fibre diet.

CHAPTER SEVEN. FAECAL BILE ACIDS

The aetiology of multiple polyps and colonic neoplasia developing from foci of epithelial dysplasia in the colon may be investigated by measuring the physical and chemical factors acting on these areas. These promoting factors are for the main part in the lumen of the large bowel, although it is impossible to exclude promoting factors that are transported by the circulation to reach the target area of dysplasia in the colon. It has been suggested that large bowel contents contain carcinogenic compounds, including those that have initiating and promoting activity (Wynder et al 1974). It has also been suggested that the faeces of high and low risk groups should be examined for the possible differences in chemical and bacterial content, since the gut bacteria may play a role in converting an active carcinogen from an inactive precursor. (Aries et al 1971 and Wynder and Reddy 1974).

Epidemiological studies indicate that diet is a major aetiological factor in bowel cancer (Correa et al 1978, Wynder and Reddy 1974). Diets high in total fat and low in fibre as well as a high intake of beef are associated with a higher incidence of colon cancer in humans (Armstrong and Doll 1975, Burkitt 1978, Correa et al 1978, Wynder 1969). To explain the relationship between dietary fat, fibre and cancer of the large bowel, it has ben hypothesised that a high fat diet not only changes the composition of bile acids but also modifies the activity of the gut microflora which produce tumour promoting substances from bile acids in the lumen of the colon (Hill et al 1971, Reddy et al 1977, Reddy 1981).

I. Materials and Methods

(a) Animals

Thirty male albino Sprague-Dawley rats weighing 200 grams were obtained from a random inbred colony. Three groups of animals were studied.

Group A: 10 rats on a high fibre diet (Oxoid 41 without antibiotics and 15% crude fibre). The rats were injected with DMH at a dose of 15 mg/kg body weight as weekly subcutaneous injections for 16 weeks.

Group B: 10 rats on a normal fibre diet (oxoid 41 without antibiotics) similarly injected with DMH as in Group A.

Group C: Control group of 10 rats on a normal fibre diet (as in Group B) and injected with the carrier substance EDTA.

Protective clothing was worn while handling the carcinogen, rats and their excreta.

(b) Procedure and Measurements

All the rats were maintained under similar conditions. The animals were allowed free access to the appropriate diet.

Total faecal bile acid excretion was measured on faecal collections. These were collected while the animals were in metabolic cages and each rat was studied for a period of 24 hours on week 1, 8, 16, 20, 24

and 28 weeks into the experiment. An aliquot of homogenised faeces was freeze dried for the assay. All analyses were carried out on 0.2 grams of freeze dried faeces. Steroids were extracted by heating with glacial acetic acid at 120°C for 60 minutes and then mixing with 10 ml of Toluene. The resulting supernatant filtered off and evaporated to dryness. The extract was then hydrolysed and redissolved in 3 ml methanol, adding 0.3 ml aq 10 N KOH and heated at 80°C for 60 minutes. The neutral steroids were then removed by extraction with petroleum ether. The bile acids were extracted from the remaining aqueous phase using chloroform. This was evaporated to dryness and redissolved in ethanol and sodium barohydride added to convert any keto acids present to hydroxyacids. The bile acids were then re-extracted after 60 minutes using ethyl acetate and the extract evaporated to dryness. The total faecal bile acids wre estimated using a hydroxysteroid dehydrogenase enzyme assay.

EXTRACTION OF FAECAL BILE ACIDS

1. A B24 tube is set up of 0.2 g of freeze-dried faeces mixed with 5 ml glacial acetic acid. A standard of 1 mg deoxycholic acid/5ml glacial acetic acid is also set up. The tubes are heated at 120°C/60 minutes.

2. 10 ml toluene is added and mixed for one minute. A clear supernatant separates off and the solids are removed by filtering. The supernatant is evaporated to dryness in a hot water bath under a stream of N_2.

3. The toluene/acetic acid extract residue is redissolved in 3 ml methanol and 0.3 ml of aq. IONKOH added. The tubes are heated at 80°C/60 minutes keeping the level of methanol above the 3 ml mark.

4. The tubes are left to cool then 2 ml H_2O is added.

5. Extract 2 x 20 ml and 1 x 10 ml petroleum ether (60-80°C) - the neutral steroid extracts are discarded.

6. Add 5 ml 20% NaCl then 3 ml 25% HCl to the aqueous layer and then extract 2 x 20 ml 1 x 10 ml chloroform. The chloroform extract containing the acid steroids is evaporated to dryness under a stream of N_2.

Preparation of HSD Assay

1. The residue from the chloroform extract is redissolved in 2 ml ethanol. A small spatula of $NaBH_4$ is added and tubes are left for a minimum of one hour.

2. Add 5 ml sat. NaCl then 5 ml conc. HCl.

3. Extract 2 x 20 ml + 1 x 10 ml ethyl acetate and evaporate the extract to dryness under a stream of N_2.

4. Redissolve in 2 ml Methanol.

Assay

Into cuvette: a) 0.1 ml sample, standard-deoxycholic acid or blank.

b) 1 ml Tris-EDTA buffer pH 9.5.

c) 1.5 ml hydrazine hydrate.

d) 0.3 ml DPN.

Mix with plastic paddle and read at 340 nm until stable. This is 001.

e) 0.1 ml HSD.

Mix with plastic paddle and continue to read at 340 nm, temp 25°C, until reaction stops. This is 002.

CALCULATION

$\triangle 00 = 00_2 - 00_1$

umoles bile salt/ml methanol $= \triangle 00$ test $- \triangle 00$BK $\times \frac{3}{6.22} \times 10 \times 2.$

Collection 1

Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs
A1	4.299	2.796	12.01	B1	4.866	4.561	22.19	C1	4.429	3.800	16.83
A2	3.968	3.820	15.16	B2	8.085	4.369	35.33	C2	4.930	3.674	18.11
A3	3.591	4.245	15.24	B3	4.422	4.561	20.17	C3	8.356	5.194	43.40
A4	3.453	4.754	16.43	B4	8.005	6.105	49.10	C4	7.420	4.875	36.16
A5	2.100	5.262	11.05	B5	4.378	3.854	16.88	C5	7.078	4.242	30.02
A6	2.765	4.329	11.97	B6	6.610	5.204	34.40	C6	3.813	3.990	15.22
A7	3.297	-	-	B7	4.888	5.404	26.41	C7	6.689	5.954	39.83
A8	3.449	0.594	2.05	B8	5.331	3.983	21.23	C8	4.002	4.560	18.25
A9	3.784	14.77	55.75	B9	3.664	10.35	37.92	C9	3.891	3.334	12.97
A10	3.703	0.119	0.441	B10	5.65	2.977	16.82	C10	5.166	-	-

TABLE X_1

FAECAL BILE ACIDS

Collection 2

Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs
A1	6.763	3.96	26.78	B1	2.111	12.375	26.13	C1	5.540	7.536	41.75
A2	3.101	6.002	11.74	B2	4.78	4.239	20.26	C2	7.336	3.203	23.50
A3	0.694	10.10	6.71	B3	7.343	8.73	42.08	C3	6.658	2.702	17.99
A4	5.193	2.491	12.93	B4	7.698	9.335	71.76	C4	3.880	6.558	25.44
A5	5.544	6.19	34.32	B5	2.647	9.118	24.14	C5	7.615	0.540	4.112
A6	3.850	2.988	11.50	B6	3.931	25.49	100.20	C6	4.559	0.463	2.110
A7	2.623	3.548	9.307	B7	4.849	9.477	45.94	C7	6.074	-	-
A8	5.537	3.164	17.52	B8	5.398	8.290	44.75	C8	7.610	1.467	11.16
A9	4.445	2.545	11.32	B9	6.963	1.516	10.55	C9	4.228	4.860	20.54
A10	4.699	1.671	7.852	B10	7.381	3.628	26.79	C10	7.036	3.770	26.53

TABLE X_2

FAECAL BILE ACIDS

Collection 3

Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs
A1	5.393	1.017	5.484	B1	6.330	5.685	35.98	C1	5.237	4.927	25.80
A2	5.535	1.895	10.49	B2	5.334	5.318	28.36	C2	7.144	2.653	18.95
A3	4.507	4.09	18.44	B3	2.680	5.685	15.24	C3	4.277	2.880	12.31
A4	5.754	1.895	10.90	B4	5.401	0.872	4.712	C4	6.636	6.629	43.99
A5	5.492	4.09	22.46	B5	5.395	4.019	21.68	C5	6.898	7.626	52.60
A6	5.516	4.242	23.40	B6	6.632	4.322	27.50	C6	7.625	6.588	50.23
A7	5.640	2.805	15.82	B7	6.993	4.094	28.63	C7	4.133	11.25	46.50
A8	5.351	4.571	24.46	B8	3.466	8.373	29.018	C8	4.395	5.541	24.3536
A9	6.321	3.503	22.52	B9	6.429	4.476	28.778	C9	6.785	7.134	48.4019
A10	-	-	-	B10	-	-	-	C10	-	-	-

TABLE X_3

FAECAL BILE ACIDS

Collection 4

Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs
A1	4.1934	7.635	32.017	B1	-	-	-	C1	4.28	7.616	32.597
A2	3.241	7.465	24.192	B2	4.082	4.463	18.216	C2	5.231	3.511	18.3685
A3	3.218	3.013	9.698	B3	4.743	10.116	47.9777	C3	4.088	7.174	29.327
A4	2.471	6.598	16.303	B4	4.14	4.638	19.2014	C4	2.685	7.554	20.2847
A5	1.513	7.534	11.398	B5	5.014	10.371	52.004	C5	2.7781	7.834	21.7637
A6	-	-	-	B6	3.595	7.67	27.573	C6	4.5925	5.951	27.3299
A7	3.709	5.60	20.774	B7	4.009	7.508	30.1036	C7	3.805	6.9676	26.8143
A8	4.013	1.157	4.643	B8	-	-	-	C8	3.590	5.314	19.0792
A9	-	-	-	B9	3.69	7.187	26.521	C9	3.602	9.401	33.8665
A10	3.009	11.05	33.25	B10	-	-	-	C10	-	-	-

TABLE X_4

FAECAL BILE ACIDS

Collection 5

Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs
A1	2.061	3.288	6.776	B1	5.653	5.514	31.171	C1	5.006	6.9676	34.8841
A2	2.906	3.225	9.374	B2	2.203	6.25	13.7675	C2	1.759	6.92	12.1783
A3	5.019	3.688	18.508	B3	6.467	7.217	46.674	C3	4.588	6.714	30.8044
A4	5.211	2.87	14.956	B4	3.941	4.199	16.5499	C4	4.477	6.785	30.3742
A5	3.287	0.199	0.677	B5	3.608	5.036	18.1687	C5	5.399	6.087	32.862
A6	5.623	1.157	6.506	B6	3.852	5.013	19.312	C6	-	-	-
A7	-	-	-	B7	-	-	-	C7	-	-	-
A8	4.166	2.204	9.192	B8	-	-	-	C8	-	-	-
A9	-	-	-	B9	-	-	-	C9	-	-	-
A10	-	-	-	B10	-	-	-	C10	5.439	4.169	22.674

TABLE X_5

FAECAL BILE ACIDS

Collection 6

Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. mg	umoles /gm	Conc/ 24 hrs	Group	Faecal Wt. gm	umoles /gm	Conc/ 24 hrs
A1	3.984	1.338	5.329	B1	3.497	7.508	26.257	C1	2.171	5.898	12.807
A2	3.482	2.448	8.824	B2	3.433	5.978	20.5234	C2	3.127	5.951	18.608
A3	5.641	0.393	2.219	B3	4.0273	7.488	30.144	C3	4.716	4.812	22.692
A4	5.317	3.019	16.054	B4	2.421	5.541	13.416	C4	5.217	4.199	21.907
A5	3.275	4.127	13.817	B5	3.329	9.321	31.029	C5	3.916	7.378	28.893
A6	4.481	2.590	11.606	B6	4.794	7.58	36.336	C6	5.792	4.743	27.488
A7	-	-	-	B7	-	-	-	C7	5.449	6.170	33.623
A8	-	-	-	B8	-	-	-	C8	-	-	-
A9	-	-	-	B9	-	-	-	C9	-	-	-
A10	-	-	-	B10	-	-	-	C10	2.035	6.012	12.235

TABLE X_6

FAECAL BILE ACIDS

II. Results

Faecal Bile Acids

The mean baseline level of micromoles bile acid of stool collection for the control rats was 23 micromoles. The levels for the subsequent collections were 17.3 micromoles, 35.9 micromoles, 25.5 micromoles, 27.3 micromoles and 21 micromoles. There was no significant difference between these results. The mean baseline levels of micromoles of bile acid per stool collection for the high fibre diet rats was 14 micromoles and for the normal fibre diet rats was 28.1 micromoles. The difference between these two results was significant but neither of these groups showed a significant difference with the controls. The levels obtained from the second and third collection (i.e. the period before tumour developed) showed a significant difference in some instances but this did not seem to follow a set pattern. During the fourth, fifth and sixth collections, tumour development had started and the levels between the two diet groups and the controls did show differences. Although there was not a significant difference in the fourth collection, in the fifth collection the difference between the high fibre diet rats and the normal fibre diet rats was significant as well as between the high fibre diet rats and the controls which were highly significant. In this collection there was no difference between the normal fibre diet rats and the controls. In the fifth collection the difference between the high fibre diet rats and the normal fibre diet rats showed a highly significant difference. This was also the case with the high fibre rats compared to the control group. There was no significant difference between the normal fibre diet rats and the control (TABLE XI).

MEAN AND STANDARD DEVIATION

umoles/24 hours

A		B		C	
$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
A 14.01010	15.95578	B 28.04500	10.69973	C 23.07900	13.72947
2A 14.99790	8.89016	2B 41.26000	26.98647	2C 17.31320	13.08229
3A 17.10822	6.82333	3B 24.43311	9.33197	3C 35.90394	15.40075
4A 19.03438	10.42191	4B 31.65657	13.30173	4C 25.45898	5.83757
5A 9.42700	5.84829	5B 24.27385	12.50018	5C 27.29617	8.48825
6A 9.54150	5.19382	6B 26.28423	8.21253	6C 20.98140	5.89131

t-test

A and B	t = 2.31023	d.f = 18	P<0.05
A and C	t = 1.36242	d.f = 18	P<0.2
B and C	t = 0.90219	d.f = 18	P<0.4
2A and 2B	t = 2.92288	d.f = 18	P<0.01
2A and 2C	t = 0.42890	d.f = 18	P<0.7
2B and 2C	t = 2.52503	d.f = 18	P<0.05
3A and 3B	t = 1.90085	d.f = 16	P<0.1
3A and 3C	t = 3.34749	d.f = 16	P<0.01
3B and 3C	t = 1.91101	d.f = 16	P<0.1
4A and 4B	t = 2.02497	d.f = 13	P<0.1
4A and 4C	t = 1.54180	d.f = 15	P<0.2
4B and 4C	t = 1.49620	d.f = 14	P<0.2

5A and 5B	t = 2.66965	d.f = 11	P<0.05
5A and 5C	t = 4.34743	d.f = 11	P<0.01
5B and 5C	t = 0.48996	d.f = 10	P<0.7
6A and 6B	t = 4.22053	d.f = 10	P<0.01
6A and 6C	t = 3.38265	d.f = 9	P<0.01
6B and 6C	t = 1.24360	d.f = 9	P<0.3
6A and 6C	t = 3.7231	d.f = 12	P<0.01
6B and 6C	t = 0.9317	d.f = 11	P<0.4

	$\bar{x}$	SD
6C	22.2816	7.5965

DAILY FAECAL BILE ACID EXCRETION

Collection (weeks)	Diet + D.M.H. High Fibre (Group A) N = 10	Diet + D.M.H. Low Fibre (Group B) N = 10	Diet Control (Group C) N = 10	Statistical Significance (P value)
1	14.0	28.0	23.1	A,B P<0.05
8	15.0	41.3	17.3	A,B P<0.01
16	17.1	24.5	35.9	A,C P<0.01
20	19.1	31.7	25.5	A,B P<0.01
24	9.4	24.3	27.3	A,B P<0.05 A,C P<0.01
28	9.5	26.3	22.3	A,B P<0.01 A,C P<0.01

TABLE XI

FAECAL BILE ACID EXCRETION VALUES IN RATS

ON A HIGH AND A LOW FIBRE DIET

III. Discussion

Faecal Bile Acid Excretion was significantly reduced in rats on the high fibre diet. The faeces of people in areas with a high incidence of colon cancer has a high bile content and a highly anaerobic bacterial flora in the colon capable of metabolising the steroids. It has been suggested that endogenous intestinal carcinogens may be formed from bile acid compound. The diet therefore affects the composition of intestinal contents and the colonic environment which is in direct contact with the bowel mucosa (Hill 1971). There are a number of bile acids that have been demonstrated experimentally to be carcinogenic (Wynder and Shigamatsu 1967, Lacassagne et al 1961). An increase in the bile acid content of the large bowel by mechanical means has been reported to enhance the carcinogen activity of azoxymethane induced tumours in rats (Chomchai et al 1974). Similarly, when cholestyramine was given to this experimental model it appeared to increase the tumour yield in the large bowel but it had no effect on the number of tumours in defunctionalised bowel of rats that had colostomies. The origin of the colonic carcinogens is probably the bacterial matabolism of bile salt. High bacteroides counts were found in stools of those people living in high cancer risk areas and a greater proportion of these bacteria could dehydroxylate bile salts. In addition their stools had higher concentrations of faecal steroids and their metabolites (Hill 1974, Reddy 1981).

A great deal of interest has been shown in the potential activity of certain bile acids and neutral sterols. They have a steric similarity to carcinogenic polcyclic aromatic hydrocarbons. The human gut flora especially the clostridia have been shown to achieve partial

aromatisation of the steroid ring (Hill 1974) and faecal concentration of bile acids, neutral steroles and bacteria are definitely diet related (Hill 1971, Reddy and Wynder 1973, 1975). The evidence for bile acids as colon tumour promotors comes from animal experiments (Chomchai et al 1974, Nigro 1973). Surgical diversion of the bile to the middle of the small intestine enhanced colon tumour formation. Taurodeoxycholic acid (TDA) and lithocholic acid (LCA) act as colon tumour promotors in experimental models (Reddy 1973). Other workers have shown that deoxycholic acid (DOA) also does this in germ-free rats (Narisawa 1974). An increased dietary fat intake was associated with an increased faecal bile acid and neutral sterole excretion (Antonis and Bensohn 1962). Some workers have found that the excretion of total bile acids and secondary bile acids such as DCA, LCA was increased in western diets (Bandaru et al 1977). These could act as colon tumour promotors in animal models and since they are present in human faeces in large amounts one could assume that they might act likewise (Reddy et al 1976, Narisawa et al 1974, Weisburger 1982*).

Faecal bacteria had a higher beta-glucuronidase activity (Reddy 1973), and since many exogenous and endogenous compounds including important cacinogen metabolites are excreted in the bile as glucuronide conjugates, the data available might be interpreted that the colonic bacteria are more active in hydrolysing these conjugates (Bandaru et al 1977). This was tested and found to be so in that the patients with colonic cancer had a higher faecal bile acid concentration and faecal clostridia which could dehydrogenate the faecal bile acid nucleus (Hill et al 1975). The activity of faecal 7-alpha-dehydroxylase which converts cholic acid and chenodeoxycholic acid to DCA and LCA was

found to be higher in patients with colon cancer (Hill et al 1975). Hence patients with colon cancer are more able to convert primary bile acids into secondary bile acids which act as colon tumour promotors in experimental animal models (Bandaru et al 1977, Hill 1975). These faecal bile acids have been tested and are carcinogenic for mouse skin (Hill et al 1974, Bachmann et al 1975) and co-carcinogenic in the mouse rectum (Wynder 1974). The microflora can play a modifying role in DMH induced carcinogenesis in the experimental model since gut bacteria are important in the carcinogenic activity of the intestinal carcinogens and germ-free animals are protected from it (Reddy et al 1974, Wiebecke 1969).

High dietary fat intake has been associated with increased faecal acid and neutral sterole excretion (Antonis and Bensohn 1962). Experimental work has shown how faecal bile acids were more extensively degraded in rats on a high fat diet and these animals were more susceptible to colon tumour induction by DMH (Reddy et al 1976, Portman and Murphy 1958). More recent studies suggest that both the high intake of total fat and the low intake of certain fibres are necessary for the full expression of risk to colon cancer (Reddy 1981). There is good epidemiological evidence now that the concentration of secondary bile acids was lower in a low risk population who had a three-fold higher consumption of fibre which was mainly cereal type than the same fibre in a New York population who are a high risk group (Reddy et al 1978). The effect of wheat bran and alphalfa in an experimental model caused a significant increase in stool weight in rats and the concentration of faecal bile acids DCA and LCA was lower in rats fed on wheat bran compared to those fed on a controlled diet (Reddy et al 1980). There

have been variations and controversies in the literature about bile acid excretion in subjects on a high fibre diet. When cellulose was added to the diet of rats it increased the faecal bile acid excretion and degredation of products (Stanley et al 1972). In man cellulose increases faecal bile acid output, but methyl cellulose is without effect. Studies with bran gave conflicting results and were unable to show that bran enhanced the excretion of bile acids (Eastwood et al 1973).

This variation in experience can be explained by the fact that conjugated bile acids in bowel and upper duodenum are weakly absorbed to fibre while unconjugated bile acids as are found in the colon are strongly absorbed. This property is pH dependent and it is greater at an acidic pH (Eastwood and Mitchell 1974). Faecal excretion of bile acid varies with the type and amount of dietary fibre. Variations in the literature are due to the different types of non-nutritive fibres and their isolated component that bind the individual bile acids differently (Reddy 1981). Some workers have shown the effects of various dietary fibres on faecal bile acid concentration and wheat bran showed the greatest reduction in change of faecal bile acid excretion on a daily basis (Eastwood and Mitchell 1974, Kay and Truswell 1977).

The high intake of dietary fibre in this experimental animal model was shown to significantly reduce the total faecal bile acid excretion on a number of occasions during the experiment. A high fibre isocaloric diet in an experimental model for multiple polyps and colonic neoplasia induced by DMH injection protects the sites of dysplasia in the

colonic mucosa. One mechanism is by reducing the amount of faecal bile acid excreted which acts as a colon tumour promotor in this model.

CHAPTER EIGHT. INTESTINAL TRANSIT TIME

Dietary fibre has an important role to play in the prevention of colonic neoplasia. The more refined the diet, the smaller the stool and the slower the intestinal transit time (Burkitt 1971). There is an inverse relationship between stool rates and transit time, the bulky stool containing more fibre weighs more and is propelled through the bowel faster. (Burkitt 1971). The length of time that dietary residues remain in the colon is important in determining colonic metabolism. It is thought to be one of the factors which affects the occurrence of colonic neoplasia (Burkitt 1971) and may be relevant to the extent to which the gut microflora metabolise foreign organic compounds (Scheline, 1973) and dietary fibre (Southgate, 1973). These noxious substances would remain in contact with the colonic mucosa for a longer time and in a concentrated form, affecting mainly the left side of the colon if there is a slow intestinal transit time as is seen in diets deficient in fibre.

In keeping with this theory that the duration that the dietary residue remains in contact with the colonic mucosa is a determining factor in the promotion of colonic neoplasm in an area of colonic dysplasia, the intestinal transit time was studied in this experiment.

I. Materials and methods

(a) Animals

Thirty male albino Sprague-Dawley rats weighing 200 grams were obtained from a random inbred colony. Three groups of animals were

studied.

Group A: 10 rats on a high fibre diet (Oxoid 41 without antibiotics and 15% crude fibre). The rats were injected with DMH at a dose of 15 mg/kg body weight as weekly subcutaneous injections for 16 weeks.

Group B: 10 rats on a normal fibre diet (Oxoid 41 without antibiotics) similarly injected with DMH as in Group A.

Group C: Control group of 10 rats on a normal fibre diet (as in Group B) and injected with the carrier substance (EDTA).

Protective clothing was worn while handling the carcinogen rats and their excreta.

(b) Procedure and measurements

All the rats were maintained under similar conditions. The animals were allowed free access to the appropriate diet.

The mean intestinal transit time was measured at 12 and 26 weeks into the experiment in order to obtain readings before and after tumour development. Under Halothane anaesthesia, 25 precision balls from RHP Precision Ball Company Ltd., size 1.0 mm, grade 100 carbon-chrome steel RHP11-03 (SAE 52100) Gauge A were syringed down a nasogastric tube (intravenous cannula 5FG O/D 1.65 mm, length 30 cm, red line 200/300/050 gamma) into the stomach. The tube was removed and on recovery the rats were fed their appropriate diet (Illustration 40).

Collection of faeces in metabolic cages over four hourly periods for 48 hours were made at 12 and 26 weeks. The faeces passed were x-rayed and the ball bearings counted. The mean intestinal transit time was taken for 50% of the ball bearings to appear after ingestion. This was measured in a collective manner for the whole animals in the different groups studied (Illustration 41).

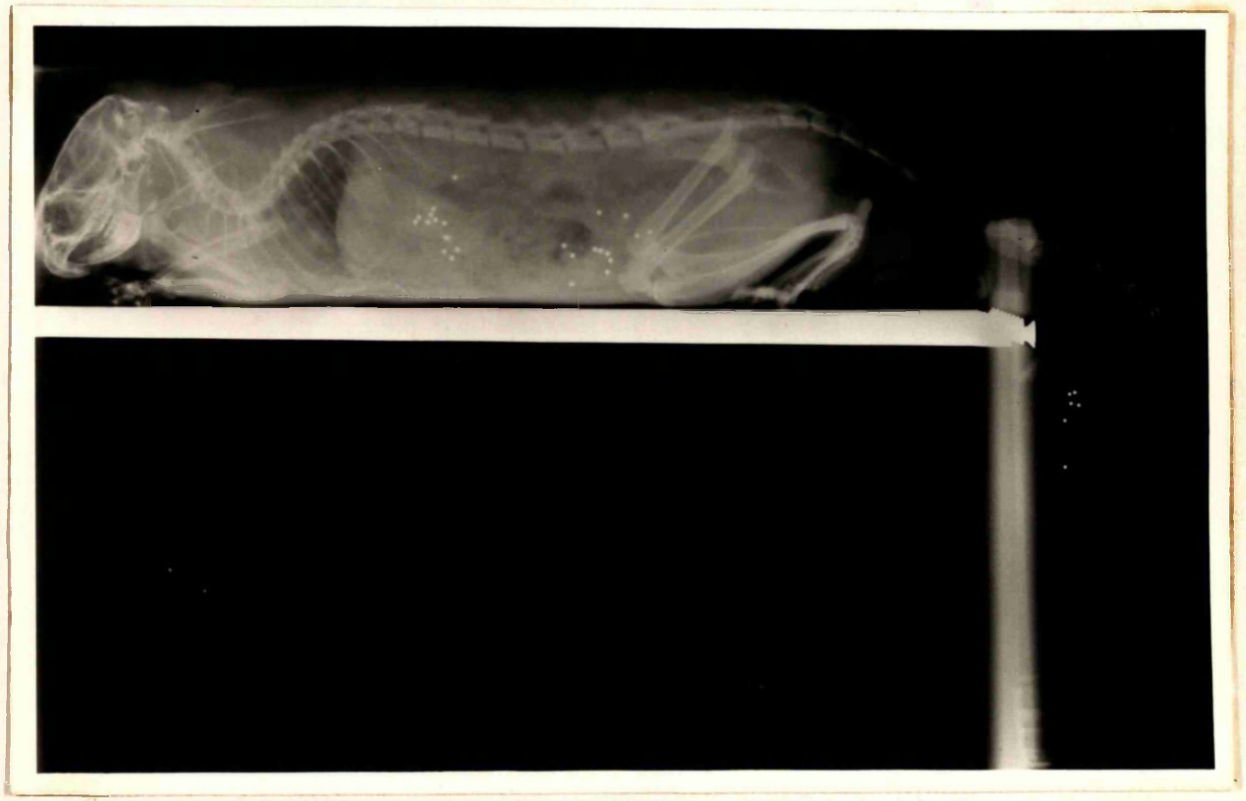


Illustration 40

X-ray of a rat after the introduction of 25 ball bearings into the stomach through a trans nasogastric tube. This x-ray demonstrates the presence of the 25 radio-opaque balls at various stages along the alimentary tract. The intestinal transit time was measured using timed faecal collections.

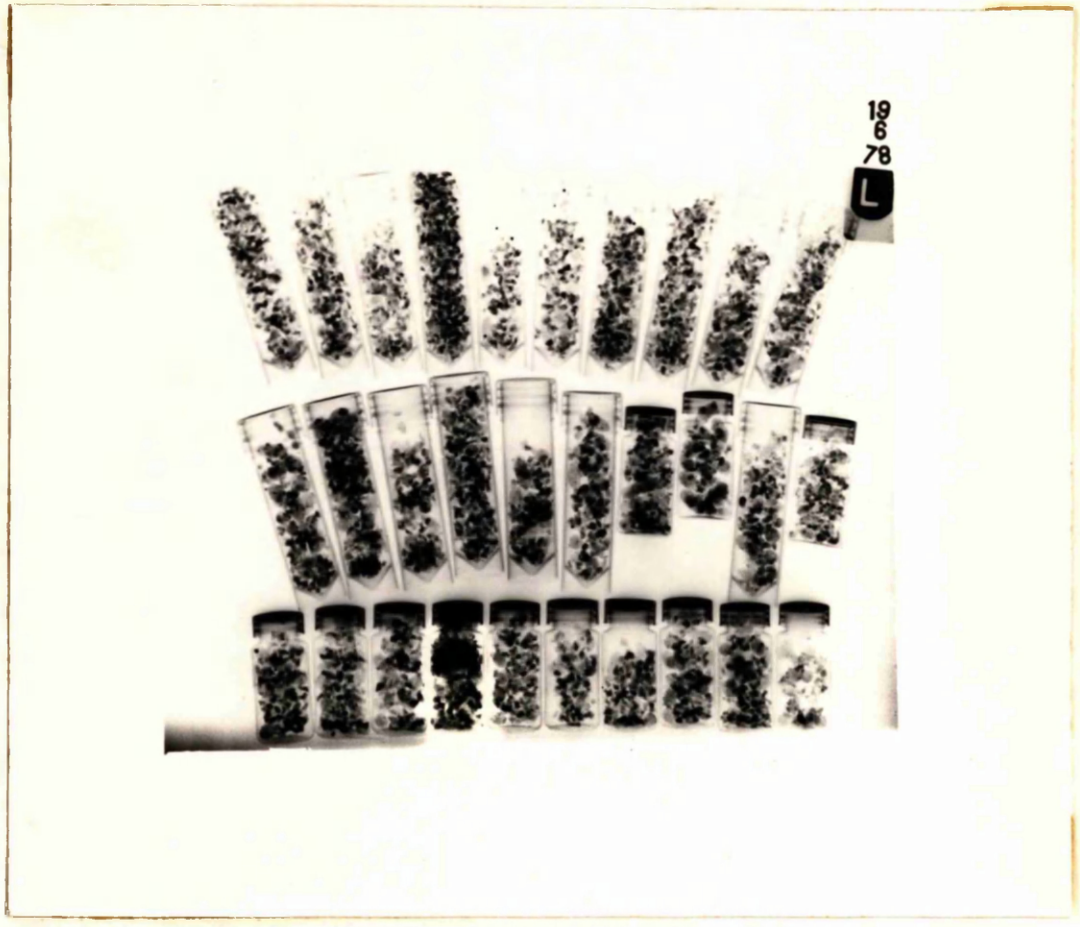


Illustration 41

Radiological appearance of a timed faecal collection of the three groups of animals: those on a high fibre diet, those rats on a low fibre diet and the controls on a low fibre normal diet who did not receive any carcinogen. The radio-opaque ball bearings are visible and can easily be counted accurately.

c Recordings

Collection 1 (Week 12)

C	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0

Collection 2 (Week 12)

C	0	6	0	0	1	0	0	0	0	0
A	0	0	18	13	0	10	3	4	0	8
B	8	0	2	2	0	0	0	0	0	3

Collection 3 (Week 12)

C	4	8	3	13	3	5	6	3	21	5
A	0	1	3	3	11	8	14	14	17	3
B	13	0	10	9	14	9	12	8	20	7

Collection 4 (Week 12)

C	10	4	0	2	3	0	0	0	3	0
A	0	0	0	0	4	1	1	0	3	2
B	3	0	5	0	0	1	5	1	0	1

TABLE XII_1

FAECAL COLLECTIONS

Collection 5 (Week 12)

C	0	1	0	0	12	0	0	20	0	0
A	19	2	0	0	6	0	0	0	4	3
B	0	16	0	0	1	8	0	1	2	0

Collection 6 (Week 12)

C	0	0	18	0	0	1	0	0	0	0
A	0	0	0	5	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0

Totals (Week 12)

C	14	19	21	15	19	6	6	23	24	5
A	19	3	21	21	21	19	18	18	24	16
B	24	16	17	11	15	18	17	10	22	11

TABLE XII_2

FAECAL COLLECTIONS

Collection 1 (Week 26) (3 animals sacrificed)

C	0	0	0	0	0	0	0	0	0	0
A	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0

Collection 2 (Week 26)

C	8	0	10	1	9	9	5			
A	0	0	0	2	0					
B	0	0	0	0	4					

Collection 3 (Week 26)

C	2	0	6	1	2	1	4			
A	0	4	8	15	13					
B	7	8	0	10	15					

Collection 4 (Week 26)

C	1	0	3	1	0	2	5			
A	0	7	2	2	0					
B	4	7	18	5	1					

TABLE XII_3

FAECAL COLLECTIONS

Collection 5 (Week 26)

C	0	0	1	3	1	0	2				
A	0	0	3	0	4						
B	0	1	0	0	3						

Collection 6 (Week 26)

C	9	20	0	14	8	8	4				
A	20	9	7	1	3						
B	9	4	2	5	0						

TABLE XII_4

FAECAL COLLECTIONS

II. Results

The mean intestinal transit time was mesured at 12 weeks before tumour developed and at this stage measurements were easy to make. By 24 hours into the collection, group A had passed 72% of the total ball bearings while groups B and C had passed 64% and 60% respectively. The mean intestinal transit time in group A was 8 hours and in groups B and C was 12 hours (Illustration 42). The decrease in intestinal transit time in the high fibre diet group A animals, was significant at a $P<0.05$.

The intestinal transit time was far more difficult to measure at 26 weeks because some animals had developed diarrhoea and early signs of intestinal obstruction, because of tumour formation. The mean intestinal transit time was 12 hours for group A and 16 hours for group B. The controls, group C, had a mean intestinal transit time of 8 hours. These results should be interpreted in the presence of a number of factors that were causing a delay in transit time which included intestinal obstruction, diarrhoea as a result of the tumour formation.

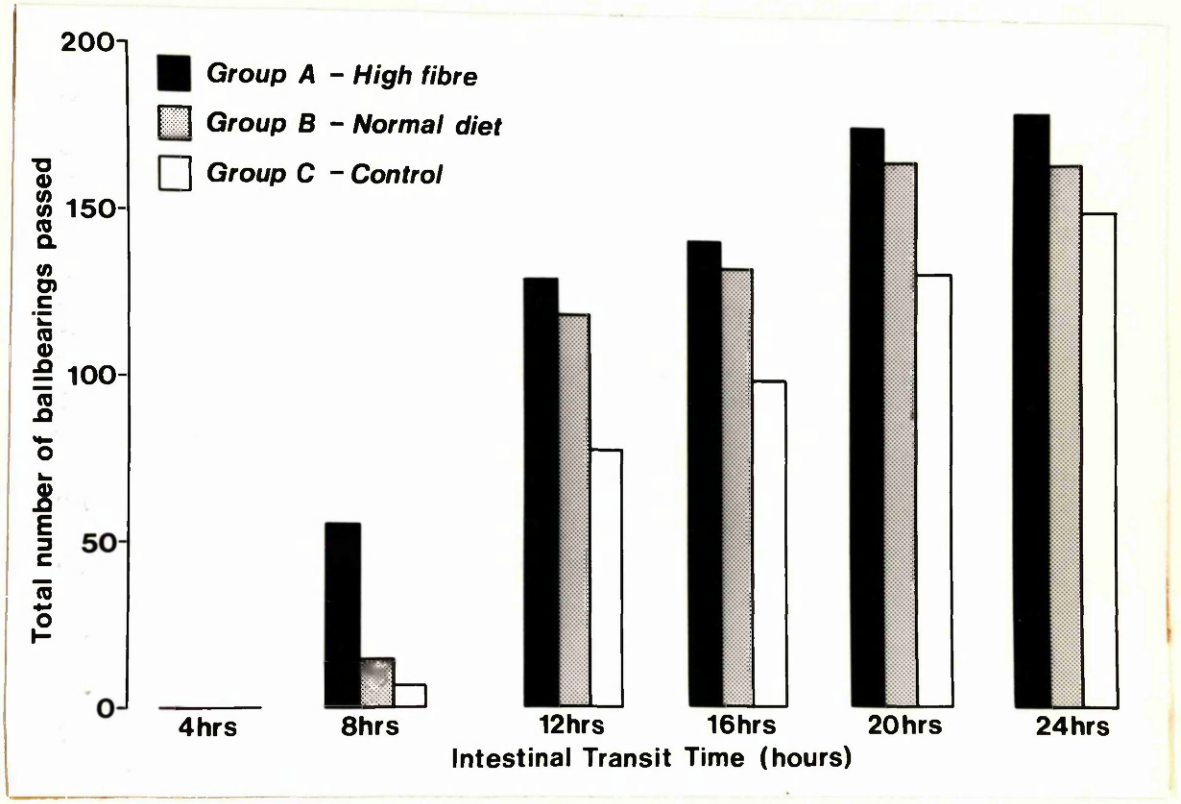


Illustration 42

Histogram illustrating the cumulative number of ball bearings passed in each of the groups at different time intervals. The rats on a high fibre diet had passed a significantly greater number of ball bearings by eight hours when compared to the rats on a low fibre diet or the control group.

Statistics at 8 hours intestinal transit time:

Group A: mean 5.6 s.d. 5.9 s.e. 1.8

Group B: mean 1.5 s.d. 2.2 s.e. 0.69

Group C: mean 0.7 s.d. 1.4 s.e. 0.47

A-B = t = 1.88 not significant

A-C = t = 2.32 significant at 5 per cent $P<0.05$

B-C = t = 0.797 not significant

III. Discussion

Dimethylhydrazine administered at low dose of 15 mg/kg body weight weekly for 16 weeks induces polypoidal disease of the colon. Rats on a high fibre diet develop fewer tumours which are better differentiated and less aggressive than rats on a normal diet (Carachi 1983). High fibre diet alters the environment of the colon and protects the unstable colonic mucosa in the experimental model by decreasing the mean intestinal transit time.

The transit time is the time taken for food residue to pass through the gut and in man this is shortened by a high dietary fibre (Cummings 1973). The effect of dietary fibre is confined to the transit of faecal residue through the large bowel since fibre has no effect on small intestinal transit rate (Hill 1974). Some workers have noted that there is an inverse relationship between the daily stool weights and transit time, the bulky stool containing more fibre weighs more and is propelled through the bowel faster (Burkitt 1971). The more refined the diet the smaller the stool and the slower the passage of the food residue through the intestine (Walker 1947, 1961, 1969, 1970). It has been postulated that fibre may protect against colonic cancer by decreasing the intestinal transit time and so allowing less time for carcinogens to be produced by colonic bacteria (Burkitt 1971). Bacteria in the colon of people eating a western diet degrade bile to form the precursors of carcinogens. These noxious compounds would remain in contact with the colonic mucosa for a longer time and in a more concentrated form, if the fibre deficient diet is eaten (Burkitt 1972).

Transit time, the time it takes for a marker to pass through the gastrointestinal tract, has been measured by a variety of techniques since the original observations by Hertz using bismuth (Hertz et al, 1907). A single dose of marker is given as coloured glass beads (Alvarez and Freedlander, 1924), carmine (Mulinos, 1935) seeds, ball bearings, (Burnett, 1923; Haelzel, 1930) radio-isotopes (Hansky and Connell, 1962), radio-opaque polyethylene pellets (Hinton et al, 1969) and a radio-telemetry capsule (Connell and Rowlands, 1960; Holdstoch and Misievwiez, 1970) or a radio-isotope capsule (Rosswich et al, 1967; Kirwan and Smith, 1974) and transit calculated from the appearance of marker on x-ray or in successive stool.

An accurate estimate of the average time a marker spends in the gut can be obtained by feeding marker continuously for several weeks and measuring the output in the stools (Dairnon et al, 1968, Cummings et al, 1965). These methods are cumbersome and may involve irradiation. A method described (Cummings and Wiggans 1976) which requires the collection of only one stool involves giving 20 radio-opaque markers on each of three consecutive days. Each marker is a different type. A simple stool transit time correlated sufficiently with the continuous marker technique ($P<0.001$).

A modification of two well described methods was used for the measurement of the intestinal transit time in this experimental model. Hinton's method was modified using 25 radio-opaque balls as a measure of the transit time and this combined with the new method to measure the mean transit time (MTT) of digestion when these pellets were fed as a single dose (Hinton 1969, Cummings 1976). The results suggest

that the single dose measurement is more accurate and reproducible than Hinton's method (Cummings and Wiggins 1976). The mean intestinal transit time was easily measured early on in the experiment when the animals had not yet developed tumour. Rats on a high fibre diet were found to have a significantly reduced intestinal transit time when compared to rats on a normal low fibre diet. This experimental evidence confirms the work reported on human subjects (Cummings and Wiggins 1976). Measurements later on in the experiment when tumours were present were marred by the presence of irregular habits in the rats, some having diarrhoea, blood in the stools and incipient intestinal obstruction from the presence of multiple polypoidal disease of the colon.

CHAPTER NINE. FAECAL RESIDUE

The amount of faecal residue is proportional to the dietary intake as well as its decomposition after digestion. This decomposition is influenced a great deal by the bacterial flora of the colon. Adding fibre containing foods to a diet will increase the faecal bulk and improve bowel habit. (Eastwood 1973). Observations on dietary intake variations in different ethnic group populations has also confirmed the differences both qualitatively and quantitatively with regard to the faecal residues (Burkitt 1971). Dietary fibre has been shown to increase stool volatile fatty acids, which are the major anions of faeces, and as their lipid solubility is low, they act as osmotic cathartics. Fibre may exert its effect on increasing faecal residue in one or all of three ways. The water absorbing properties of fibre may act as a simple bulking agent, and hence promote colonic peristalsis and faster transit. The main product of bacterial metabolism of fibre is the volatile fatty acids and may increase the faecal weight in this way. Bile acids may bind on to fibre and are then released in the colon once the fibre is metabolised by the colonic microflora, and act further as a cathartic to promote discharge of faecal residue (Weisburger 1981*).

I. Materials and methods

(a) Animals

Thirty male albino Sprague-Dawley rats weighing 200 grams were obtained from a random inbred colony. Three groups of animals were studied.

Group A: 10 rats on a high fibre diet (Oxoid 41 without antibiotics and 15% crude fibre). The rats were injected with DMH at a dose of 15 mg/kg body weight as weekly subcutaneous injections for 16 weeks.

Group B: 10 rats on a normal fibre diet (Oxoid 41 without antibiotics), similarly injected with DMH as in Group A.

Group C: Control group of 10 rats on a normal fibre diet (as in Group B) and injected with the carrier substance EDTA.

Protective clothing was worn while handling the carcinogen, rats and their excreta.

(b) Procedure and measurements

All the rats were maintained under similar conditions and were allowed free access to the appropriate diets. The animals were injected each week for the first sixteen weeks of the experiment. Three metabolic cages were used for this experiment, the cages were referred to in Chapter one. Each cage housed four rats, of each group. The faecal pellets were collected at the end of a grid at the bottom of the metabolic cage and so allowed separation of urine from faeces. 24 hour collections were made and the faecal pellets were weighed expressing the result in grams of faeces excreted per rat in 24 hours. In a similar fashion the faecal pellets were placed in a volumetric cylinder and the volume of the faecal residue was expressed in cubic centimetres per rat per 24 hours.

In order to ensure that the rats in each group ate the whole quantity of the diet set for them, the food residue was measured and returned with the next feed. This was in fact rarely the case.

The measurements were to be carried out at 12 weeks when dysplasia had occurred but not yet evidence of neoplasia and at 26 weeks after the development of the tumours.

c Recordings

Control Faecal Weights (Grams) at week 12 per 24 hours			Volume (c.c.) per 24 hours		
Group C	Group A	Group B	Group C	Group A	Group B
13.0	39.0	31.0	12	35	30
21.5	25.5	29.5	18	25	26
20.5	30.0	15.5	18	30	12
22.5	43.0	26.5	18	38	25
17.5	24.0	21.0	16	25	16
25.0	23.0	17.5	22	40	16
15.5	30.0	16.5	18	25	18
21.5	36.5	25.0	21	35	20
16.5	27.5	27.5	15	30	25
15.5	36.0	18.5	17	35	18

TABLE XIII

FAECAL WEIGHTS AND VOLUMES IN RATS ON A HIGH AND A LOW FIBRE DIET

II. Results

The total faecal residue was measured in terms of weight and volume. The faecal weight was expressed in grams per 24 hours. After 12 weeks, group A excreted 31.45 grams per rat, group B 22.85 grams per rat and group C 18.9 grams per rat. These results were found to be statistically significant at a level of $P<0.01$ when comparing group A with group B and group A with group C. There was no significant difference between group B and group C (Illustration 43). The collections at 26 weeks were 23.6 grams for group A, 10 grams for group B and 25.85 grams for group C. Group B proved to be extremely difficult to collect because of the presence of diarrhoea and blood. Faecal volume was measured in cubic centimetres per 24 hours and at 12 weeks group A had a volume of 31.8 cc per rat, group B had 20.6 cc per rat and group C had 17.5 cc per rat. These results were statistically significant at a level of $P<0.01$ when comparing A with B and A with C. They were not significant when one compared group B with group C. At 26 weeks the volume of group A was 31.8 cc, group B was 17.5 cc. The results at this stage were false because of the difficulty in the collections already mentioned (Illustration 44).

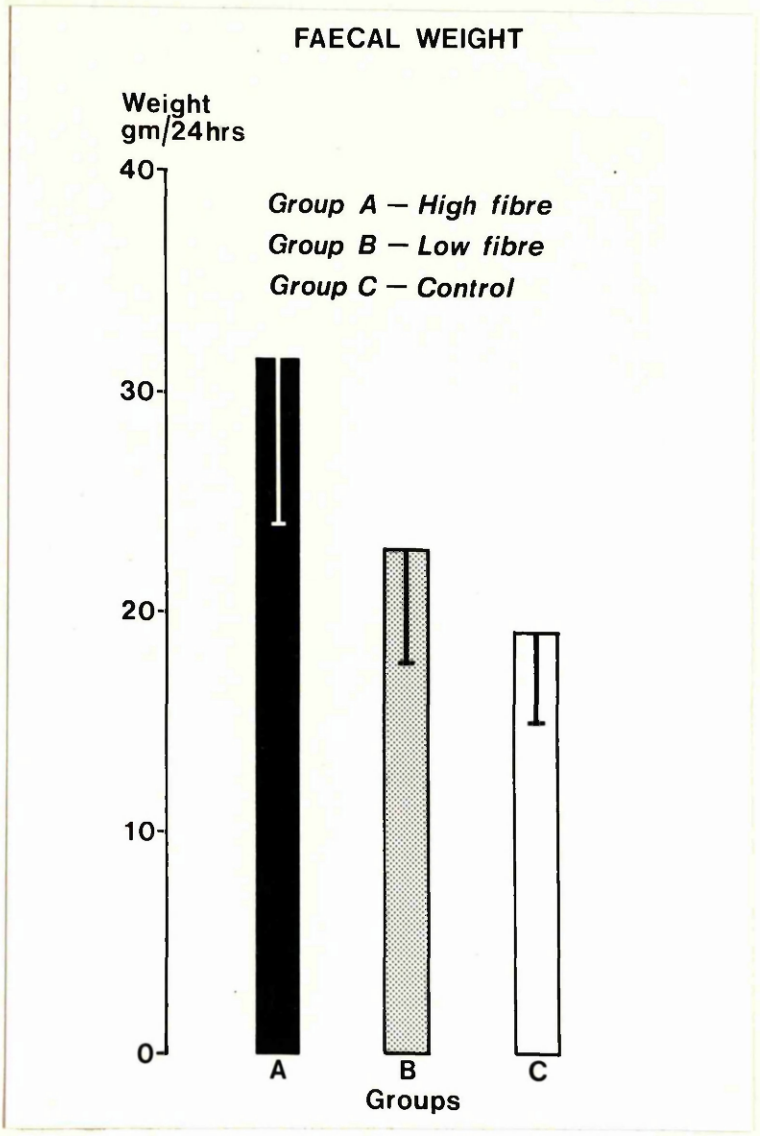


Illustration 43

The faecal weight was measured in the three groups and was expressed as grams excreted in 24 hours. The rats on a high fibre diet had a significantly greater faecal weight than the rats in the other two groups.

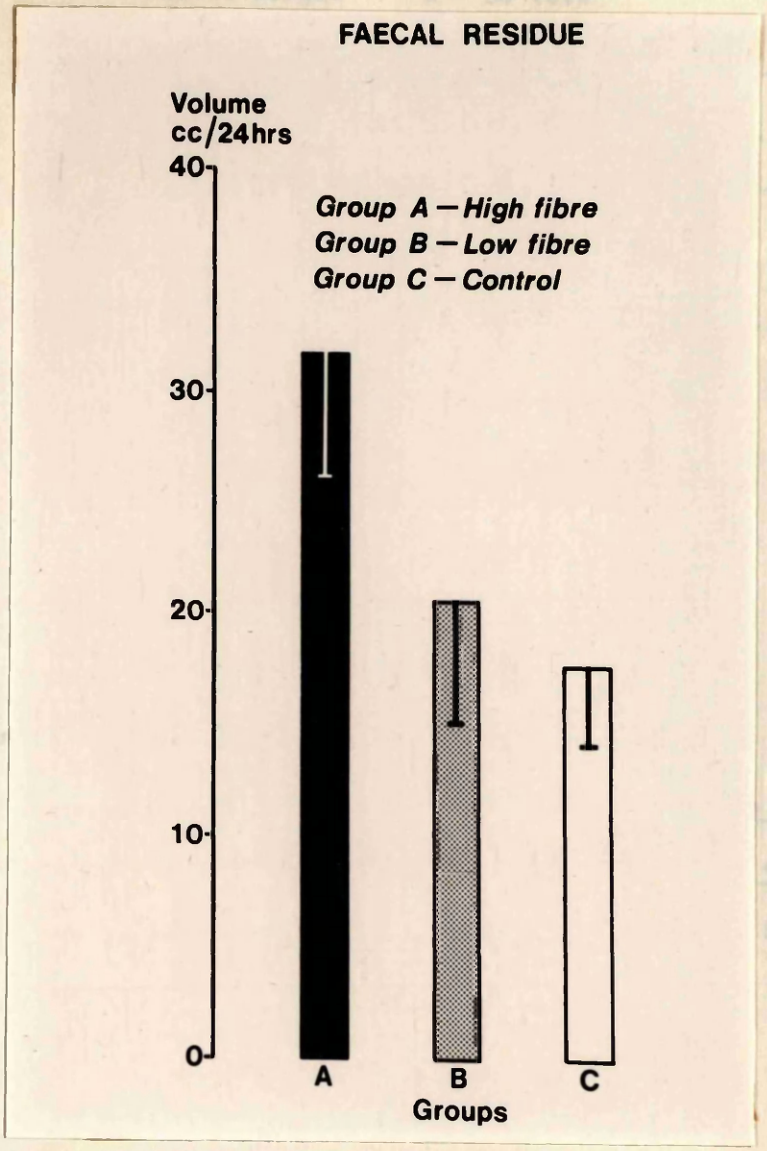


Illustration 44

The faecal volume was measured as an index of the faecal residue. The rats on a high fibre diet had a significantly higher faecal residue than the rats in the other two groups.

Faecal Weight N = 30 rats

	Group A	Group B	Group C
12 weeks	31.45 g ± 6.39	22.85 g ± 5.21	18.9 g ± 3.49
	S.E. 2.02	S.E. 1.60	S.E.1.10
26 weeks	23.6 g	10 g*	25.85 g

* Difficulty because of diarrhoea and blood.

A - B t = 3.08 Significant 1%

A - C t = 5.073 Significant 1%

B - C t = 1.84 Not significant

Faecal Volume cc/24 hours N = 28 rats (2 died)

	Group A	Group B	Group C
12 weeks	31.8 cc ± 5.2	20.6 cc ± 5.2	17.5 cc ± 2.4
	S.E. 1.60	S.E. 1.60	S.E. 0.37
26 weeks	31.8 cc	17.5 cc	

A - B t = 4.45 Significant 1%

A - C t = 7.20 Significant 1%

B - C t = 1.55 Not significant

III. Discussion

Rats on a high fibre diet develop fewer tumours which are better differentiated and less aggressive than rats on a normal diet. High fibre diet alters the environment of the colon and protects the unstable colonic mucosa in the experimental model by decreasing the mean intestinal transit time, reducing the amount of faecal bile acid excreted and increasing the total faecal weight and the faecal volume (Weisburger 1982*, Carachi 1983).

Faecal Bulk, Faecal Weight and Faecal Volume

Rats on a high fibre diet had a greater faecal weight and faecal volume than rats on a normal diet. This was confirmed for measurements both before and after tumours were induced. The high fibre diet altered the environment of the colon by increasing the total faecal weight and faecal volume in these experimental animals. The length of time that dietary residue remains in the colon is considered to be important in determining colonic metabolism. The absence of a high dietary residue is thought to be one of the factors which affects the occurrence of large bowel cancer (Burkitt 1971). The importance of the faecal stream has been demonstrated experimentally (Steele 1981, Campbell 1975). Sterilisation of the gut and rerouting the faecal stream through colostomies reduced considerably the incidence of induced colonic tumours in this model (Steele et al 1981).

The volatile fatty acids (acetic, butyric and proprionic acids) are the major anions of faeces and at the colonic pH they are all ionized, and cannot be absorbed, acting as osmotic cathartics. These fatty

acids control the faecal bulk in people (Wrong et al 1965). Dietary fibre increased the stool volatile fatty acid output at the same time as increasing the faecal weight. The origin of volatile fatty acids in stool is probably bacterial metabolism of unabsorbed carbohydrates. Dietary fibre itself is a ready source of volatile fatty acids since a significant proportion of ingested fibre is metabolised. Dietary fibre may be protective against colonic carcinogens by its water-absorbing properties, where it increased the bulk of the stools and aids in propelling faecal residue and reducing the transit time. It may also act by increasing the presence of volatile fatty acids which in turn adds to the faecal bulk and promotes a rapid transit time. The increase in bulk may help to dilute the concentration of the carcinogen in the colonic environment and may mop up carcinogen ions by acting as an ion-exchange resin (Fordtram 1971, Cummings 1978*).

CHAPTER TEN. NEOPLASIA INDUCED IN THE EXPERIMENTAL MODEL FOR COLONIC DYSPLASIA (II)

The experiments measuring the faecal bile acids (Chapter 7) the intestinal transit time (Chapter 8) and the faecal residue (Chapter 9) on the groups of rats fed off diets of a high and low fibre content involved invasive techniques which might alter the development of colonic polyps and colonic neoplasia from areas of dysplasia induced by Dimethylhydrazine (DMH).

I. Materials and methods

(a) Animals

30 male albino Sprague-Dawley rats weighing 200 grams were obtained from a random inbred colony. Three groups of animals were studied.

Group A: 10 rats on a high fibre diet (Oxoid 41 without antibiotics and 15% crude fibre). The rats were injected with DMH at a dose of 15 mg/kg body weight as weekly subcutaneous injections for 16 weeks.

Group B: 10 rats on a normal fibre diet (Oxoid 41 without antibiotics) similarly injected with DMH as in Group A.

Group C: Control group of 10 rats on a normal fibre diet (as in Group B) and injected with the carrier substance (EDTA).

Protective clothing was worn while handling the carcinogen rats and their excreta.

(b) Procedure and measurements

All the rats were maintained under similar conditions. The animals were allowed free access to the appropriate diets. The three groups of animals were placed in metabolic cages at 12 weeks and 26 weeks in the experiment, they also underwent ether anaesthesia and had nasogastric tubes passed for the purpose of the intestinal transit time experiment. The weights of the animals and their general condition was recorded weekly and they were sacrificed at 28 weeks. Following a full autopsy, the whole colon was opened and pinned out on cork board to facilitate the examination. The number and the anatomical location of the tumours was noted, as well as to whether they were plaques, sessile or polypoidal in shape. The tumours were individually dissected off the colon and were weighed using a microbalance.

Light microscopy of the lesions was performed after haematoxylin and eosin staining of the sections. The tumours were graded as either well differentiated, or anaplastic carcinomas. The regional lymph nodes were also examined after dissection for evidence of tumour metastases. Histology was also obtained of the gastrointestinal tract, and the abdominal viscera.

c Recordings

Lesion	Groups		
Plaque (dysplastic) 	A (H.F.) 10 3	B (N.F.) 10 3	C (C) 10 0
Sessile (adenomatous) 	12	1	0
Polypoid (adenocarcinoma) 	5	53*	0
Total Number	20	57	0
Total Tumour Weight	1.41296gm	9.65815gm	0

(A x B = P value< 0.01)
* = liver metastases

TABLE XIV

An analysis of the neoplsia induced, showed an equal number of plaque lesions in Groups A and B. There were more residual adenomata in Group A than Group B, and whereas there were only 5 polypoidal lesions in Group A, there were 53 induced in Group B. The total tumour weight in Group A was 1.41296 gm, compared to 9.65815 gm in Group B. These results were highly statistically significant at a P value < 0.01.

II. Results

A total of 77 lesions were induced in the colon and rectum of the experimental animals. No tumours were found in the caecum, and the tumours induced were predominantly left sided. The number of colorectal tumours induced in each rat was constant and many of these tumours were found in clusters (TABLE XIV).

Histopathology

The tumours classified as plaques showed only dysplastic changes, whereas the sessile adenomas histologically ranged from simple adenoma to carcinoma in situ. The commonest lesion was the polypoidal adenocarcinoma and many of these tumours showed invasion of the stalk which was the cause of an intussusception and haemorrhage. There was no difference in the number of dyplastic areas in Groups A and B, while there were 12 adenomas induced in Group A compared to only one in Group B. Thee were only 5 polypoidal adenocarcinomata in rats in a high fibre diet compared to a total of 53 tumours induced in rats on the normal diet. The types of tumour induced were found to be more aggressive in this latter group and two of these rats were found to have hepatic metastases.

The colonic tumours were dissected out from the colon and the total tumour mass was obtained from the two groups. A total of 1.4 grams of tumour was induced in rats on a high fibre diet compared to a total of 9.6 grams in rats on a normal diet. These values were statistically significant at $P<0.01$. Group C were free of any tumours which might have developed spontaneously from the carrier substance used, or they might have developed from the diet they received.

III. Discussion

Dimethylhydrazine administered at a low dose of 15 mg/kg body weight weekly for 16 weeks induces polypoidal disease of the colon (Carachi et al 1980). Rats on a high fibre diet develop fewer tumours which are better differentiated and less aggressive than rats on a normal diet (Carachi et al 1983). High fibre diet alters the environment of the colon and protects the unstable colonic mucosa in the experimental model by decreasing the mean intestinal transit time, reducing the amount of faecal bile acid excreted, and increasing the total faecal weight and the faecal volume.

The high intake of dietary fibre in this experimental animal model was shown to significantly reduce the total faecal bile acid excretion on a number of occasions during the experiment. The number of tumours induced in the rats on a high fibre diet was significantly smaller in this group. Similarly the total weight of tumours induced was 1.4 grams compared to 9.7 grams tumour weight in those rats on a normal low fibre diet. In conclusion a high fibre isocaloric diet in an experimental model for polyposis coli and colonic neoplasia induced by DMH injection protects the sites of dysplasia in the colonic mucosa.

This experiment has confirmed the data presented in chapter six, where it was demonstrated that dietary fibre has a protective effect on the development of colonic polyps and colonic neoplasia from dysplasia induced by the carcinogen dimethylhydrazine dihydrochloride. It has also excluded the possibility that the interference in the experiments for measuring faecal bile acids, intestinal transit times, and faecal residue had any influence on the development of the tumours.

CHAPTER ELEVEN. POLYAMINES AS TUMOUR MARKERS IN THE MODEL (II)

The group of polycationic compounds called polyamines include Spermine, Spermidine and Putrescine. Interest in the role of polyamines in tissue growth was stimulated by the observation that spermidine in rat liver increases after partial hepatectomy (Dykstra and Herbst 1965). In this system the rate of polyamine accumulation closely correlates with the increased rate of synthesis of RNA. Cell proliferation also occurs in cancer and the levels of polyamines associated with malignancy have been examined (Russell 1971, 1975, Lipton et al 1975). There was reported an eightfold increase in polyamines in a 24 hour urine collection in patients with several forms of cancer. These observations and subsequent work with improved analytical systems led to the postulate that polyamines could be predictors of success or failure in cancer chemotherapy; Spermidine could serve as a marker of tumour cell kill and putrescine reflected the proliferative behaviour of the tumour (Russell and Russell 1975). The aim of the work reported here was to evaluate measurements of urinary polyamine levels in the detection and assessment of colorectal cancer. Despite the prevalence of colorectal cancer only isolated reports of polyamine levels associated with this disease have been made (Lipton et al 1975). The polyamine levels of a group of patients with advanced colorectal cancer was assayed. Experimental animals in which colonic polyps and neoplasia were induced also had polyamine levels assayed. This experimental model was chosen because it would permit studies of tumour mass in relation to polyamine excretion.

I. Clinical material and methods

(a) Subjects

Twenty-four hour urine collections were made from 10 controls. Patients with urinary tract infection were excluded from the study as well as those with cellular proliferative disorders. The collections were made pre-operatively in patients who are admitted to a general surgical ward for elective surgical procedure, e.g. ingrowing toe-nails, varicose veins, gall stones.

There were 10 patients with colorectal cancer; 5 of these patients had grossly elevated carcinoembryonic antigen levels with clinical evidence of metastatic disease. The carcinoembryonic antigen was measured by a radioimmune assay technique. All the patients with colorectal cancer had the disease confirmed at operation and on histological examination of the tumour.

(b) Procedures and measurements

Urine samples were collected in bottles containing toluene (10 ml) and were stored at $-20^{o}C$ prior to analysis.

II. Experimental material and methods

(a) Animals

Seven male Sprague-Dawley rats with a starting weight of 150 g received 1,2 Dimethylhydrazine dihydrochloride subcutaneously at a dose of 15 mg per kg body weight on a weekly basis for 16 weeks. Four rats acted as controls and received subcutaneous injections of carrier

substance (EDTA).

(b) Procedures and measurements

The rats were placed in metabolic cages and 24 hour urine collections were made at 26 weeks. Urine samples were pooled for groups of 3 or 4 rats, acidified to pH3 and stored frozen prior to analysis. The experimental animals were sacrificed at 30 weeks to determine the number of tumours induced, as well as the total tumour weight.

Polyamine Assay

Urine samples (5 ml) were mixed with Aristar grade concentrated HCl and hydrolysed for 18 hours at 110°C in evacuated sealed Pyrex tubes to release conjugated polyamines. After removal of acid on a rotary evaporator the dried residue was redissolved in 1.5 ml of 0.2 M sodium citrate buffer pH2.2 and centrifuged to remove particulate material. A sample (1 ml) was analysed on a Locarte amino acid analyser using a column (0.9 cm x 3 cm) packed with Locarte High Grade ion exchange resin. Elution was at a flow rate of 30 ml/hour with a buffer programme in which 3 citrate buffers pH5.28, 0.35M, 1.35M and 2.35M (with respect to sodium) were pumped in turn for 20 minutes, 60 minutes and 170 minutes. The column was regenerated by washing for 70 minutes with 0.2M NaOH and then re-equilibrated with 0.35M sodium citrate pH5.28 for 70 minutes. Column operation was at 55°C. Analysis of the eluate with ninhydrin was started 80 minutes after the sample was applied; this avoided an excessively high baseline. Recrystallised standards of the hydrochlorides of putrescine, spermidine and spermine were employed.

Subject	Urine vol ml	Putrescine	Spermidine	Spermine	Total	C.E.A.	Diagnosis
1.	1140	2.0	1.2	0.3	3.5	>100	Ca. colon
2.	1110	2.5	1.4	0.5	4.4	30	Ca. colon
3.	1140	3.9	2.6	<0.1	6.6	25	Ca. colon
4.	915	1.4	1.1	<0.1	2.6	23	Ca. colon
5.	1102	0.9	1.0	<0.1	2.0	0	Ca. colon
6.	1035	1.5	1.2	0.3	3.0	58	Ca. colon
7.	1700	0.7	0.9	0.3	1.9	>100	Ca. colon
8.	2120	1.2	1.2	0.5	2.9	50	Ca. rectum
9.	3145	0.9	0.3	<0.1	1.3	15	Ca. rectum
10.	2085	1.8	1.7	0.1	3.6	>100	Ca. rectum
Mean ± SD		1.68±0.96 (SD)	1.37±0.72 (SD)	<0.2	3.18±1.51 (SD)		

TABLE XV_1

POLYAMINE EXCRETION mg/24 HOURS IN COLORECTAL CANCER

Subject	Urine vol ml	Putrescine	Spermidine	Spermine	Total	Diagnosis or state
1.	915	0.9	0.9	<0.1	1.9	Gall stones
2.	1420	1.4	1.2	0.1	3.7	Hiatus hernia
3.	1640	1.8	0.5	<0.1	2.4	Gall stones
4.	1820	1.5	0.9	0.1	2.5	Gall stones
5.	2500	1.1	0.5	<0.1	1.7	Gall stones
6.	755	3.0	1.6	0.2	4.8	Anal fissure
7.	840	0.4	0.3	<0.1	0.8	Ingrowing toenail
8.	1980	0.8	0.8	<0.1	1.7	Varicose veins
9.	1000	1.1	1.5	<0.1	2.7	Author (R.C.)
10.	2230	1.7	1.6	0.3	3.6	Author (J.G.B.)
Mean ± SD		1.37±0.71	0.98±0.48	<0.2	2.58±1.18 (SD)	

TABLE XV_2

POLYAMINE EXCRETION mg/24 HOURS IN CONTROLS

Group	Number of rats	Putrescine	Spermidine	Spermine	Mean Tumour No/rat	Tumour Wt/rat
Experimental 1	4	295	281	106	7.25	1.27
Experimental 2	3	213	195	51	1.70	0.05
Control	4	241	271	110	0	0

TABLE XV_3

POLYAMINE EXCRETION (ug/24 HRS/ANIMAL) IN EXPERIMENTAL ANIMALS

III. Results

Clinical Patients

The total polyamine excreted in milligrammes per 24 hours in patients with colorectal cancer was 3.18 ± 1.51 (SD). The putrescine level was 1.68 mg/24 hours ± 0.96 (SD). Spermidine was 1.37 mg/24 hours ± 0.72 (SD). The spermine levels in all patients were close to the limit of detection by the analytical procedure emloyed and the mean value was less than 0.2 mg/24 hours. Five patients had carcinoembryonic antigen levels which were grossly elevated, corresponding with the clinical disease. (Normal range CEA < 25 µg/l) (TABLE XV_1).

There were 10 control patients, eight of whom were undergoing elective surgery for gall stone, hiatus hernia, anal fissure, ingrowing toe-nails and varicose veins. The total polyamine excretion was 2.58 mg/24 hour ± 1.18 SD). The putrescine excreted was 1.37 mg/24 hours ± 0.71 (SD). The spermidine level was 0.98 mg/24 hours ± 0.48 (SD). Once again the level of spermine was barely detected, being less than 0.1 mg/24 hours (TABLE XV_2)).

Although the results showed a marginal elevation in the total polyamines excreted in 24 hours in patients with colorectal cancer, there was no statistical significance seen on testing these against the controls (Students t-test).

Animal Experiments

A total of 34 colonic polypoidal tumours were induced which were well differentiated adenocarcinoma. The total tumour weight was 5.24 g. The

controls were perfectly healthy and had no colonic tumours induced spontaneously (TABLE XV_3).

IV. Discussion

Oncofoetal antigens have not lived up to initial expectations of their suitability as screening agents for malignant disease. In particular, carcinoembryonic antigen is not always a reliable marker in relation to colorectal cancer. Of the 10 patients examined in the present study only 5 had significantly elevated levels of CEA.

Interest in polyamines was stimulated by the fact that this group of polycationic compounds are intimately related to RNA synthesis (Dykstra and Herbst 1965) and hence the synthesis of protein, one of the basic components of tumour cells.

These small organic cations may play regulatory roles in the control of growth processes. The biosynthetic pathway in bacteria has been elucidated and polyamine biosynthesis has been linked to RNA metabolism. In particular the relationship between spermine synthesis, and RNA synthesis was noted. The uptake of putrescine and its conversion into spermidine in partially hepatectomised rats is the major event. Histidine decarboxylase forms histamine, a diamine which is important in rapid cell growth and Ornithine decarboxylase is the first enzyme in the polyamine biosynthetic pathway. Ornithine decarboxylase activity is low in the liver of normal rats (200-400 CPM) (Tabor and Tabor 1964).

Counts for the first 24 hours in regenerating hepatectomised rats

livers was 20,000 CPM. Its increased activity up to 25-fold parallels the early increase in RNA synthesis and precedes the DNA by hours in regenerating liver. Similarly a close relationship was found between ornithine decarboxylase and the initiation of rapid growth in chick embryos and tumours. Putrescine synthesis is under strict modulation. The rapid turnover rate of hepatic ornithine decarboxylase is the most striking way of modulation, such that polyamine synthesis is a finely modulated process. If polyamine biosynthesis is necessary for growth processes then growth hormones increases a there are no growth processes that occur without prior stimulation of polyamine biosynthesis, (Tabor and Tabor 1964). Spermidine has no effect on RNA polymerase. In the mammalian system there are no known pathways for polyamine degredation and so all are excreted. The lower limit of detection by the electrophoretic method is 2.5 mg of amine per 24 hours. However separation of the polyamines with an amino acid analyser (Russell 1975) by a new method using gas chomotography allows the detection of even lower amounts of polyamines in normal volunteers and small amounts of all polyamines are characteristic components of human urine. A fast growing rat hepatoma with a doubling time of two days has a high spermidine/ spermine ratio while a slow growing one with a doubling time of 24 days has a low spermidine/spermine ratio. Putrescine is present only in trace amounts in slower growing hepatomas, but is present in considerable amounts in fast growing hepatomas. Relatively effective chemotherapeutic agents decreased polyamine levels in tumour systems (Tabor and Tabor 1964).

Polyamines are present predominantly as conjugates which produce the three polyamines after hydrolysis. Acidilated polyamines are the only

conjugates which have been identified in human urine. On the other hand intracellular polyamines are present predominantly in the free form. The theory that intracellular conjugation is the major route of elimination of the polyamines from cells and that the tumour cell has a higher spermidine in acetylase activity than the normal cell has been postulated. The aim of this study was to examine urinary polyamine levels as potential markers for colorectal cancer. This possibility was suggested by the finding that polyamine synthesis increases in association with cellular proliferation and by the finding of marked increases in polyamine excretion in some other cancers (Russell 1975). The polyamines putrescine, spermidine and spermine were measured in 24 urine collections prior to operation on 10 patients with colorectal cancer. Carcinoembryonic antigen assays were also peformed on these patients in an attempt to correlate these values with polyamine excretion. Polyamine excretion was also measured by use of an amino acid analyser on a group of 10 control subjects. The total polyamine levels in patients with colorectal cancer was 3.2 $\pm$ 1.5 (SD) mg/24 hours and 2.6 $\pm$ 1.2 (SD) mg/24 hours in the controls. Five of the 10 patients with colorectal cancer had elevated carcinoembryonic antigen levels.

Urinary polyamines were also measured in an experimental animal model for colorectal cancer. Measurements were carried out at 26 weeks in the experiment when a total of 34 tumours were induced in 7 experimental rats at a total tumour weight of 5.24 g. Four control rats were also used.

Although the mean 24 hour polyamine excretion was marginally elevated

in patients with colorectal cancer the difference between this group and the controls was not statistically significant. The experimental model for colorectal cancer similarly showed only marginal differences in polyamine levels between animals with extensive tumours and controls. These findings suggest that urinary polyamine measurement is unlikely to be a useful procedure to assess tumour cell mass in patients with colorectal cancer and that urinary polyamine levels are unlikely to be useful markers for human colorectal cancer.

Preliminary studies with a group of animals in which colorectal tumours or polyps had been induced by injection of dimethylhydrazine led to a similar conclusion to the studies with human clinical samples. A very slight increase in mean polyamine levels was apparent when a group of animals with high mean tumour mass were compared to the control group but the controls' polyamine values were higher than those from a group of rats with lower incidence of tumours or polyps.

The analytical system employed in these studies was an automatic amino acid analyser. This procedure is considerably more specific and gives much higher recoveries than the paper electrophosis method on which some of the earlier claims for the value of polyamine analysis in cancer were based (Russell 1971).

It is now clear that in addition to their association with cellular proliferation polyamines may be influenced by several other factors. These include variation in food intake, simultaneous infection and bacterial contamination of urine (Tabor and Tabor 1964). The latter could be eliminated if polyamines are measured in the blood.

Experimental work in this area may yet prove them to have some value as diagnostic or prognostic tools in oncology (Savory et al 1979).

Recent polyamine research has shown that ornithine decarboxylase activity and DNA synthesis was stimulated by bile acids in rat colon explant culture. Induced rat colonic polyamine biosynthetic enzymes like ornithine decarboxylase by bile salts may be related to colon carcinogenesis up to three months after DMH treatment (Takano 1981*, 1982*).

CHAPTER TWELVE. GENERAL DISCUSSION AND CONCLUSIONS

THE CLINICAL RELEVANCE OF THE EXPERIMENTAL MODEL

This thesis is based on an experimental model for colonic dysplasia with a natural history progressing to multiple polypoidal colon cancer. The clinical relevance of this model is based on those precancerous conditions of the colon which give rise to dysplasia. These precancerous states which develop into colonic polyps and neoplasia are defined as states which predispose towards the development of malignancy although not neoplastic in itself. It is not known whether all dysplastic states irreversibly progress to invasive carcinoma although the risk must be very high indeed.

The persons at increased risk from colorectal cancer are those patients with the genetically predetermined condition of familial polyposis, members of colorectal families, certain patients with a long history of ulcerative colitis, patients with ureterosigmoidostomies, and a large proportion of patients with adenomas of the colon and rectum.

Epithelial dysplasia is the common histological and cytological precursor change for all these groups, and the magnitude of risk varies with the degree of epithelial dysplasia, as well as other environmental factors.

The concept of epithelial dysplasia as the precursor lesion for colorectal cancer in man is new, but there is evidence that it is the

best nomenclature for describing these precancerous lesions in the large bowel.

COLONIC CARCINOMA IN CHILDREN

It is accepted that young people who have ulcerative colitis are liable to develop cancer of the colon. Seven children with ulcerative colitis who developed cancer of the colon before 16 years of age have been reported (Wilcoks and Beattie 1956). Malignant changes can occur in early life in Familial Polyposis; cases of carcinoma occurring in children under 16 years of age are documented by various authors (Gross 1953, Swenson 1958, Stowens 1959 and Holt, Mackintosh and Barnett 1962). Indeed the patient reported by Holt and others was age five when the cancer was noted. A carcinoma of the colon was reported in a new born premature infant, with multiple anomalies (Ahfeld 1880) and others described an adenocarcinoma of the colon in a nine month old girl (Keen and White 1958). Most authors comment on the increased incidence of colloid carcinoma in these children; this is a type with a high grade of malignancy with early metastases and high mortality rate. Approximately 50% of the cases of colonic cancer in childhood were colloid tumours as compared to the 5% in the adult population (O'Brien 1967). Anaplastic lesions are also commoner in children (Pemberton 1970). The commonest situation for carcinoma in this age group is in the sigmoid colon, and the distribution corresponds to that found in adults. In the series reviewed there were twice as many boys as girls (Dukes 1947).

Genetic observations; Familial cancer of the colon occurring without

polyposis is now authenticated. The pedigree of a family in which 11 out of 24 members in three successive generations were affected by solitary or multiple malignant lesions, and who died at an average age of 44 years was recorded (Mathis 1962).

CANCER FAMILIES/COLON CANCER SYNDROMES:

There is some indication of a familial tendency in patients with precancerous polyps (Wolff et al 1955) and established cancer (Mathis 1962, Kluge 1964, Lynch and Krush 1967) which suggests that genetic factors could be involved. Relatives of patients who have had intestinal cancer, or precancerous polyps are the persons presumed to be more at risk than the general population. The scientific evidence for a genetic factor in the cause of the intestinal cancer and precancerous polyps is insufficiently strong at the present time to justify raising unnecessary fears among the relatives of the propositi. There are a group of syndromes which are well recognised clinical entities and have been listed by the geneticists.

Similar families affected with (familial non-polypotic) carcinoma of the colon have been detailed and monozygotic twins 40 years of age in whom carcinoma of the rectum developed in identical situations has been recorded (Klug 1964, Aure and Nillson 1965, Pltokallio 1966, Fielding 1969, and Protic and Pric 1960). Apart from what can clearly be described as familial carcinoma of the colon, the statistical investigations (Wolff 1958 and Macklin 1960) lend support to the hypothesis that the genetic component exists for cancer of the large intestine distinct from that of multiple polyposis. Close relatives of

patients with carcinoma of the large bowel have a significantly higher incidence of colorectal malignancy than the control, being about three times that expected. Colorectal cancer (non-polypotic) develops at an earlier age among relatives of patients of colonic carcinoma than in the relatives of patients without malignant disease; this was particularly marked in patients with multiple carcinomas (Moretel 1958).

Heredity may be of importance because surveys have shown that children of parents with intestinal cancer had a 3 and 1/2 times the expected rate themselves. It is also a well established fact that some families are unduly prone to develop cancer of the colon.

The major criteria for a 'cancer family' have been laid out. A minimum of 25% of 1st degree relatives have a histological evidence of cancer. The lesions involve multiple anatomical sites. The onset of the malignancy is at an early age. Several generations are affected. Segregations are found. Carcinoma of the colon, as well as of the endometrium, and multiple primary malignancies are found more frequently than in the general population.

Syndrome	Mode of inheritance	Anatomical Location & pathology of polyps or cancer or both	Associated clinical features
Familial polyposis coli	Autosomal dominant	Colon, adenomatous polyps	None
Gardner's syndrome	Autosomal dominant	Colon, adenomatous polyps; adenomatous polyps may occasionally be found in the small intestine and stomach	Soft tissue (sebaceous cysts fibromas) and bone lesions (osteomas of mandible, sphenoid and maxilla), rare occurrences thyroid carcinoma and retro-peritoneal sarcoma.
Tucot's syndrome	Autosomal recessive	Colon and central nervous system; adenomatous polyps	None
Peutz-Jegher's syndrome	Autosomal dominant	Entire gastrointestinal tract, except esophagus, harbours polyps which may show malignant degeneration cancer issue remains controversial in spite of recent documen-tation), hamartomas of muscularis mucosa, ovarian tumours.	Melanin spots of oral and vaginal mucosa and distal portions of fingers
Solitary polyps	Autosomal dominant	Colon	None
Ulcerative colitis	Possible autosomal dominant in certain families	Colon	Occasionally arthritis, systemic manifestations, and psychological aber-rations

cont'd.....

TABLE XVI

Syndrome	Mode of inheritance	Anatomical Location & pathology of polyps or cancer or both	Associated clinical features
Juvenile polyposis coli	Autosomal dominant	Hamartomatous polyps of colon; increased occurrence of adenomatous polyps and adenocarcinoma in relatives; connective tissue abnormality	None
Cancer family syndrome	Autosomal dominant	Colon, endometrium, other adenocarcinomas	Various adenocarcinomas, particularly of endometrium; multiple primary malignant neoplasms; early age at onset of cancer
Generalized gastrointestinal juvenile polyposis	Not established	Stomach, small and large bowel	None
Generalized gastrointestinal adenomatous polyposis	Possible autosomal, dominant	Stomach, small and large bowel; adenomatous polyps	Desmoid reported
Cronkite-Canada syndrome	No known familial reports	Stomach, small and large bowel; adenomatous polyps	Skin pigmentation, atrophy of nails, hypoproteinemia
Familial combined breast and colon cancer	Possible autosomal	Colon and breast	None

TABLE XVI

FAMILIAL CANCER SYNDROMES (Lynch et al.)

Catalogue of Colon Cancer Syndromes and/or Tumour Associations
Showing Familial or Genetic Aggregations in Certain Families

CARCINOMA OF THE COLON ARISING AT URETERAL IMPLANT SITES

Ureterosigmoidostomy has been performed as a palliative procedure for carcinoma of the genitourinary tract or as part of a reconstructive procedure. It is performed in infants and children with a view to providing lifelong internal reconstruction. The development of the iatrogenic cancer at this site gives rise to great concern. To date more than 30 cases have been described where carcinoma has developed after a ureterosigmoidostomy for benign conditions. Colon carcinoma develop at the site of reimplantation in approximately six per cent of patients who undergo ureterosigmoidostomy for benign conditions (Schipper and Decter 1981).

Several factors have been identified as possible agents or obligate co-factors in the induction of the carcinoma. If identifiable, alterations in the surgical procedure and further understanding of the aetiology of colonic neoplasia may be gained. Factors which are inducers of colon cancer, or co-inducers are the stool, urine, colonic mucosa and ureteral mucosa, suture material at the anastomotic site, chronic irritation of the anastomotic area. Observations suggest that the ureter and faeces in the sigmoid colon environment serve as adequate co-inducers of colonic cancer.

POTENTIATION OF DMH BOWEL CARCINOGENESIS IN RATS

Rats underwent connection of the urinary and faecal streams (ureterosigmoidostomy), and with a proximal faecal diversion. The incidence of adenocarcinoma of the colon adjacent to the junction

between the bladder and the bowel was significantly higher in animals after ureterosigmoidostomy compared with animals whose faecal stream had been diverted (Steele 1981).

COLONIC POLYPS

Cancer in the intestine mostly presents in the form of one or more polyps. This is a purely clinical term used to describe any tumour projecting from the surface of the intestinal mucous membrane, whether sessile or pedunculated (Morson 1962). There are many different types of intestinal polyps, which vary greatly in their clinical significance. It is only the adenoma, papillary adenoma and villous papilloma which are precancerous of the whole spectrum (TABLE XVII). Those polyps that result from an inflammatory process, and the hamartomatous polyps such as the juvenile and the Peutz Jeghers types in all probability have no malignant potential. It is well known that the patients with the neoplastic types of polyps are more prone than the general population to develop such tumours (Wolff 1955), as well as intestinal cancer (Kirsner 1960). Patients who have had partial removal of the intestine for colorectal cancer are at risk from the development of entirely new tumours in residual large bowel. It has been calculated (Bussey and Mason 1967) at least 3.5% will develop a secondary primary growth on the average eight years after the operation. Moreover those patients who had adenomatous polyps as well as cancer in the first operation specimen stand more than twice the chance of developing a further primary tumour than when no polyps are seen initially.

In a proctoscopic study of 5000 people without any lower bowel disorder, no less than 6% were found to have polyps, and 0.5% to have carcinoma (Bussey 1970). Benign polyps of the large bowel are seen commonly in the later age groups. Clinical studies show that 80% to 90% of pedunculated adenomas occur in the rectosigmoid region, but autopsy studies show a different incidence. Half occur in the ascending and transverse colon, and only 30% are found in the rectum and rectosigmoid.

Type	Solitary	Multiple
neoplastic	adenoma papillary adenoma Villous papilloma	Familial adenomatous polyposis (syn, polyposis coli)
hamartomatous	juvenile Peutz Jeghers	juvenile polyposis Peutz Jeghers
inflammatory	benign lymphoid polyp	benign lymphoid polyposis inflammatory polyposis in colitis
unclassified	metaplastic	multiple metaplastic polyps

TABLE XVII

CLASSIFICATION OF POLYPOIDAL LESIONS OF THE LARGE INTESTINE

(MORSON 1968)

Ulcerative colitis may be complicated by the development of cancer of the colon. There is general agreement that the incidence of super-veningcancer is between 3 per cent to 5 per cent of all cases (Edwards and Truelove 1964). It has also been estimated that the risk of cancer patients with ulcerative colitis is between 5 to 10 per cent of the general population (Goldgraber and Kirsner 1964). Cancer is likely to develop in the patient who has had symptoms approximately 10 years, and more likely in those where the disease starts early in life. It has been confirmed that patients with total colitis are more at risk while the distal form of the disease have an almost insignificant risk factor. Patients with symptoms of chronic continuous disease seem to be more prone to cancer than those with the acute relapsing type. In 2000 cases of ulcerative colitis reviewed by the Mayo clinic, 5 per cent had cancer supervening. The cumulative risk of developing cancer of the colon was calculated for a group of 250 patients seen at the Radcliffe Infirmary, Oxford, and is twelve times after twenty years following operation.

While malignant change is a well established feature of colitis, it is doubtful if it can be caused by Crohns' disease. Although cancer of the colon and rectum are occasionally seen in Crohns' disease, it occurs in the cancer age group, and the association may be just one of chance.

The polyp-cancer sequence is controversial, even though one out of 3 of all operated specimens for cancer of the colon and rectum will contain one or more benign neoplastic polyps. In the conditions of familial polyposis in which neoplastic polyps of the colon are found

in the second or third decade, carcinoma arises in a much younger age group than in the general population.

Size is an important consideration in the malignant potential of polyps since the frequency with which invasive carcinoma is found increases with the size of the polyp. Polyps under 1 cm. in diameter are only very rarely found to contain invasive carcinoma.

POLYPOSIS:

Familial polyposis also known as polyposis coli and adenomatous polyposis is due to a dominant gene which may be passed on by affected persons of either sex to their children (Veal 1965). The manifestation of the penetrance rate is about eighty per cent. It has been estimated that one in five of propositi is the result of a mutation as there is no family history. Familial polyposis is a disease where hundreds of adenomatous polyps appear in the colon and rectum. There is a dramatic decrease of malignant disease among call up relatives (14%) as compared with the group presenting with symptoms (67%). The majority develop polyps in adolescence rarely earlier, but a minority show no evidence of disease till middle age. There are 260 families with polyposis coli and 818 affected members on the St. Mark's registered list (1982). Polyposis has been reported at an earlier age in a few instances, and parents are warned that intestinal symptoms in the child should be looked into immediately.

It is rare for this condition to begin after the age of forty. Solitary casesmay arise in families, and these are due to mutations.

The risk of cancer is very great in affected individuals. A report of 1200 members of polyposis families showed that 234 of them were known to have suffered from polyposis, and 167 had developed intestinal cancer (Dukes 1956). The condition is characterised by hundreds or even thousands of polyps, but never less than 200, throughout the colon and the rectum. However they are concentrated in the rectum, and are always visible, by sigmoidoscopy. Sizes vary from 1mm - 4cm.

Since a dominant autosomal gene transmits the disease, it is essential to draw up a complete family tree. About 20% of these patients have no family history of polyposis, and are considered to be mutants. Theoretically, when one parent is affected, only 50% of the children should develop the disease. In practice, the manifestation of the penetrant trait is only about 80% of this, i.e. the number of cases seen is fewer than the number expected.

There are probably 6 genetically distinct varieties of intestinal polyposis, the commonest being the one limited to the colon, which in its full blown stage is densely carpeted with polyps. Malignant degeneration is a notorious feature of this disease. It is transmitted as an autosomal, non sex linked dominant trait. On the average half of the offspring are affected regardless of sex. In any series of cases, the early and more severely affected members, may be mutations, and handed down this dominant trait. Familial polyposis tends to show the pseudo phenomenon, since some affected persons succumb to colon cancer by the age of 20 while others live to reproduce. It is suggested that the linkage exists between the familial polyposis locus and the M.N. blood group system locus. That this happens, means that the two loci

are on the same pair of chromosomes (Veale 1965).

The numbers of intestinal adenomas present in individual patients varies considerably, from the solitary tumour to many thousands. The incidence of patients with 10 or 20 adenomas is small and probably less than 1 per cent of the total. Those who in their lifetime produce more than 20 adenomas fall into two sharply divided groups. The first and smaller group between 20 and 70 adenomas in the large intestine., the second and larger group upward of 100 adenomas in the large intestine, which approach the familial polyposis patient. The question arises whether the first group also have polyposis coli. It has been suggested that in addition to the gene causing familial polyposis (P) there may also be a gene (p) for adenoma production in these patients with a much less degree of penetrance so that fewer tumours result in the phenotype. The adenoma producing gene being recessive. Thus only cases with the genotype (pp) will have adenomas.

Secondary bile acids in duodenal bile and colonic absorption of deoxycholate were significantly higher in polyposis coli patients. This suggests that bacterially degraded bile salts are involved in the adenoma-carcinoma sequence of the colon (Van der Werf et al 1982*).

THE PROBLEM OF COLORECTAL CANCER:

Colorectal cancer is one of the commonest forms of malignant disease. The death rate in England and Wales is second only to lung cancer, and gastric cancer deaths are a close third on the list. There are 5500 deaths per annum in England from rectal cancer, and 9000 from cancer of the colon. The combined incidence is rising (Registrar General Statistics Cancer Supplement).

The death rate of colorectal cancer, has seen little overall change over the same period. The number of new cases registered each year has remained constant over the past few years for England, Scotland and Wales.

The incidence of intestinal cancer increases with age. The average age at the time of diagnosis being around 60 years.

Colonic cancer is much less common in Japan than in the USA but rectal cancer has a similar incidence in the two countries. It has been shown that patients with colonic cancer have a higher socioeconomic status and eat a more westernised diet than rectal cancer patients. It was shown that the high incidence in Europe and the low incidence in rural Africa, Asia and Japan is due to diet differences (Burkitt 1971). Races who eat bulky high residue diet have a low incidence of cancer while if they emigrated or changed their diet, they developed cancer more readily. The aetiology suggested, is that ingested carcinogens remain in contact with the large bowel mucosa for longer periods. The possibility being that diet influences the pathogenesis of colonic cancer by altering the bacterial flora of the gut. The faecal bacterilogy of British and Ugandan adults was compared, and the faeces of the British has more bacteroides and bifidobacteria, but fewer streptococci and lactobacilli than those in Ugandan Africans. Bacteroids are known to be very active in the degredation of bile salts, and may produce carcinogens (Aires 1969). The following observations strongly suggest that boewel cancer is related to the diet (Burkitt 1971). The diet is the most important factor affecting the environment of the bowel. Epidemiologically bowel cancer is closely associated with highly processed foods being rare in all less

developed communities. Bowel cancer is rare in all animals and the gastrointestinal tract and the bronchi are the only epithelial lined ducts highly prone to cancer, and they are the only ones in which we can alter the contents. Bowel carcinogens given to experimental animals give rise to no tumours in a loop of bowel surgically isolated, and so no longer conveying faecal matter (Steele 1981).

A relationship between bowel cancer and cellulose deficiency has been demonstrated; (Burkitt 1971) (Illustration 7). A diet rich in unabsorbable roughage passes more rapidly through the bowel and produces large, soft stools with little odour. A low residue diet, causes concentration of any possible carcinogen, and a longer transit time in the colon for it to act on the colonic and rectal mucosa. Possible relationship between bowel cancer and bacterial flora has been shown from the following facts. When carcinogens capable of causing cancer are given orally to experimental animals, their maximum effect is normally in the proximal bowel, whereas the maximal incidence of bowel cancer in man is in the distal colon where bacterial proliferation is maximal. Tumours are rare in the small intestine, which has rapidly passing liquid and relatively abacterial contents. Carcinogens which produce intestinal tumours in normal rats are ineffective if rats are kept in a sterile environment with resulting bacteria free faeces. Epidemiologically bowel tumours are related to western diets, which are known to alter the bacterial flora of the gut. Bacteria can cause the degredation of normal bile salts to form carcinogens in vitro. A greater proportion of the bile salts can be recovered from faeces of the Bantu living on a high residue diet in South Africa than from the faeces of Europeans on a more refined

diet. This suggests that part of the bile salts of the latter has somehow been disposed of, possibly by bacterial action.

Alterations in diet from the traditional patterns to the more highly processed foods, especially changes in the carbohydrate content alter the bacterial flora in the gut. This could lead to the degredation of bile salts and so the formation of carcinogens. With a cellulose depleted diet and resultant colonic stasis, such carcinogens in the faeces would not only be concentrated in the smaller faecal mass, but would be in contact with the mucosa for a prolonged period. Thus the potential activity of such carcinogens might be enhanced by a combination of these factors (Cummings 1978*, Weisburger 1982*).

The high intake of fat may be significant from an aetiological point of view. However, statistically there is a definite association with obesity. The association of intestinal cancer with constipation, smoking of cigarettes or alcohol excess is very slight indeed. There is positive evidence of a familial tendency for cancer of the colon and rectum, which in some families may be associated with the prior development of adenomatous polyps. It is possible that ingested carcinogens play some part in the development of human large intestinal cancer, since growths are most common in those sites where faeces become increasingly concentrated, and remain in contact with the mucosa for the greatest length of time (Reddy 1981).

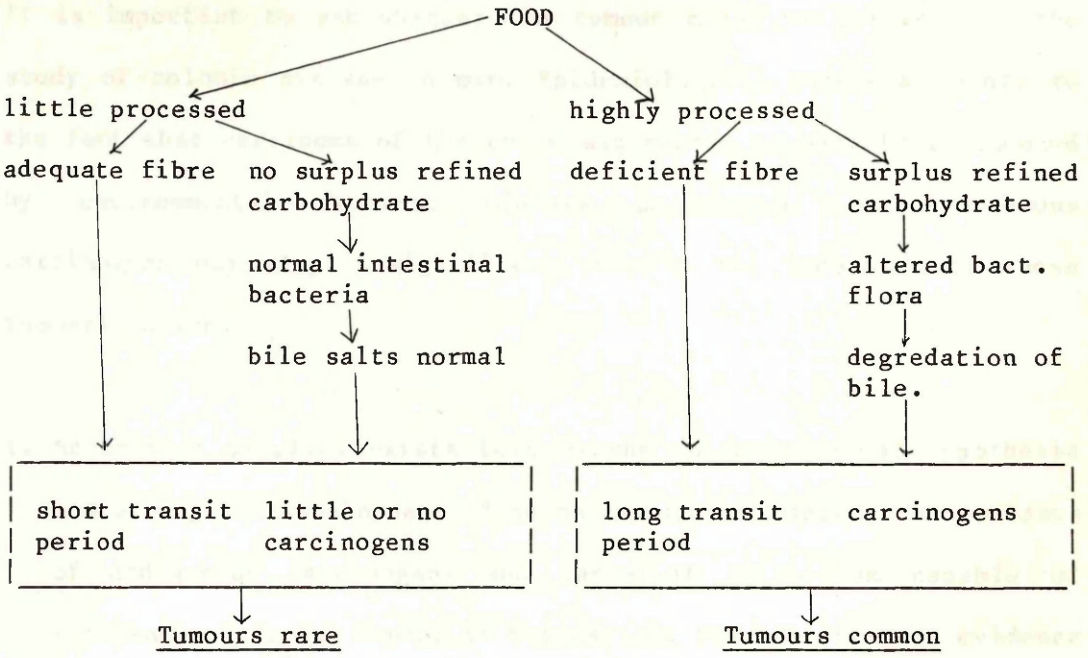


Illustration 7

* Diagrammatic representation of possible relationship between diet and cancer of the bowel (Burkitt 1971).

It is important to ask whether the tumour model is relevant to the study of colonic disease in man. Epidemiological evidence points to the fact that carcinoma of the colon and rectum is heavily influenced by environmental factors. Dietary carcinogens or endogenous carcinogens may play a significant role in the induction of these tumours in man.

1. An initial parallel exists between the epidemiological hypothesis concerning the development of human colorectal cancer, the presence of endogenous carcinogens and bacterial metabolism capable of inducing colorectal cancer in humans, and the experimental evidence for these requirements in DMH carcinogenesis in the rat model.

2. Further evidence of analogy is that both human and DMH induced carcinogens exists in the cell kinetics in both. Autoradiographic changes reflecting a decrease in the number of mucus secreting cells, inhibition of differentiation of basal epithelial cells, with an increase in the number of DNA synthesizing cells in the profliferative zone of the crypts of Lieberkuhns (Springer 1970). These findings extended for the length of the villi showing a high proliferative activity. Polyps developed in those areas closely related to the proliferative activity of the epithelium have shown similar changes, resulting in the 'net cell retention' in the mucosa of human colonic neoplasms (Deschner 1971).

3. Histopathology of the carcinogen induced tumours is comparable to the human counterpart in polyposis coli (Carachi et al 1980).

4. Evidence for the presence of oncofoetal antigens detectable by immunodiffusion, byradioimmunoassay, or by microcytotoxic assay have been demonstrated in both human and DMH induced colon cancers (Steele 1974).

5. The morphology and the natural history of the disease in the experimental animal is a close parallel to what happens in clincial practice for patients wiht colonic dysplasia undergoing malignant change.

The incidence of spontaneous colon cancer in rats is 0.1% (Miwa et al 1976 and Presean et al 1973). The discovery of the carcinogenic effect of cycasine and its aglycone on intestinal mucosa by Laqueur opened the way for research work on new synthetic intestinal carcinogens (Laqueur 1965). Druckrey demonstrated that the synthetic dialkyl-hydrazines were organotropic for gastrointestinal mucosa (Druckrey et al 1967). The important considerations for developing a standard experimental model include the type of carcinogen, the mode of administration, the dose and the species of animal used. Most of the carcinogens except for dimethylhydrazine and its metabolites azomethane and methylazoxymethane produce a low incidence of intestinal adenocarcinomas and the induction of extra intestinal tumours is common (Narisawa et al 1971).

In this model, a low dose of carcinogen was used which was sufficient to produce a fixed number of dysplastic areas in the colon in each rat. The withdrawal of the carcinogen then allowed these lesions to develop into adenomatous polyps and then carcinoma with a minimal side effect from drug toxicity. This model is a reliable and easily reproducible method for tumour induction within 20 weeks (Carachi et al 1980). The preponderance of polypoidal tumours arising from multiple foci of dysplasia bears some resemblance to patients with genetically unstable colonic mucosa who develop polyposis coli.

The colonic mucosal environment is determined by the conditions prevailing intraluminally and experimental evidence in animals with colostomies show that they develop fewer tumours in the defunctioned bowel than they did in intact animals in the same bowel segment

(Campbell et al 1975). The results show that the faecal stream alters the carcinogenic activity of the compound in the large bowel of the rat (Druckrey 1972). This model was used to study the effect of altering the colonic environment using an isocaloric high fibre diet. There was a significant decrease in the number of tumours induced in the rats on a high fibre diet.

The preponderance of left sided lesions in rats who have tumours induced by this method can be explained by the increased concentration and duration of contact by the carcinogen on the colonic mucosa. The protective role of the fibre in the diet may be due to a number of factors acting directly or indirectly affecting the intestinal transit time, the faecal bulk and the faecal bile acid production.

INTESTINAL TRANSIT TIME

The transit time is the time taken for food residue to pass through the gut, and in humans this is shortened by the high fibre diet (Cummings 1973). The more refined the diet, the smaller the stool and the slower the passage of the food residue through the intestine (Walker 1961). It has been postulated that fibre may protect against colonic cancer by decreasing the intestinal transit time and so allow less time for environmental carcinogens to be in contact with colonic mucosa (Burkitt 1971). Rats on a high fibre diet had a significantly reduced intestinal transit time compared to rats on a normal low fibre diet.

FAECAL BULK, FAECAL WEIGHT, AND FAECAL VOLUME

The high dietary residue is one of the factors which is thought to

affect the occurrence of large bowel cancer (Burkitt 1971). It may be protective against colonic carcinogens by its water absorbing properties where it increases the bulk of the stool and aids in propelling faecal residue as well as reducing transit time. It also increases volatile fatty acids which add to the faecal bulk and promotes a rapid transit time. The increase in bulk may help to dilute the concentration of carcinogens in the colonic environment and may absorb carcinogen ions by acting as an ion-exchange resin deactivating them (Fordtram 1971).

Rats on a high fibre diet had a greater faecal weight and faecal volume than rats on a normal diet. This was confirmed for measurements both before and after the tumours were induced.

FAECAL BILE ACIDS

Faeces of people in areas with a high incidence of colon cancer have a high bile content and a highly anaerobic bacterial flora in the colon, capable of metabolising endogenous bile acids to form endogenous intestinal carcinogens (Hill et al 1971). A number of bile acids have been demonstrated experimentally to be carcinogenic (Wynder 1967 and Lacassagne 1961). Similarly an increase in the bile acid content of the large bowel by mechanical means has been reported to enhance the carcinogen activity of tumour induced in rats (Chomchai et al 1974). The total faecal bile acid excretion was significantly reduced in rats on a high fibre diet, yet another mechanism that could be protecting them from developing neoplasia.

The number of tumours induced in the rats on a high fibre diet was

significantly smaller. A high fibre isocaloric diet in an experimental model for polyposis coli and colonic neoplasia induced by dimethylhydrazine protects the sites of dysplasia in the colonic mucosa.

The mechanisms outlined are: (1) The reduction of faecal bile acid excretion which acts as a colon tumour promoter in this model; (2) By increasing the total faecal bulk and diluting the carcinogen excreted in the bile; and (3) By the more rapid elimination of toxins and co-carcinogens through shortening the mean intestinal transit time that these substances have to come into contact with the colonic mucosa.

Further support for this concept has now been recorded by other workers who have used this model for testing tumour induction at the sites of ureterosigmoidostomy (Steele et al 1981).

This experimental model has clinical relevance to those pediatric patients with unstable colonic epithelium and may benefit by this experimental work in an attempt to change the pattern of their disease.

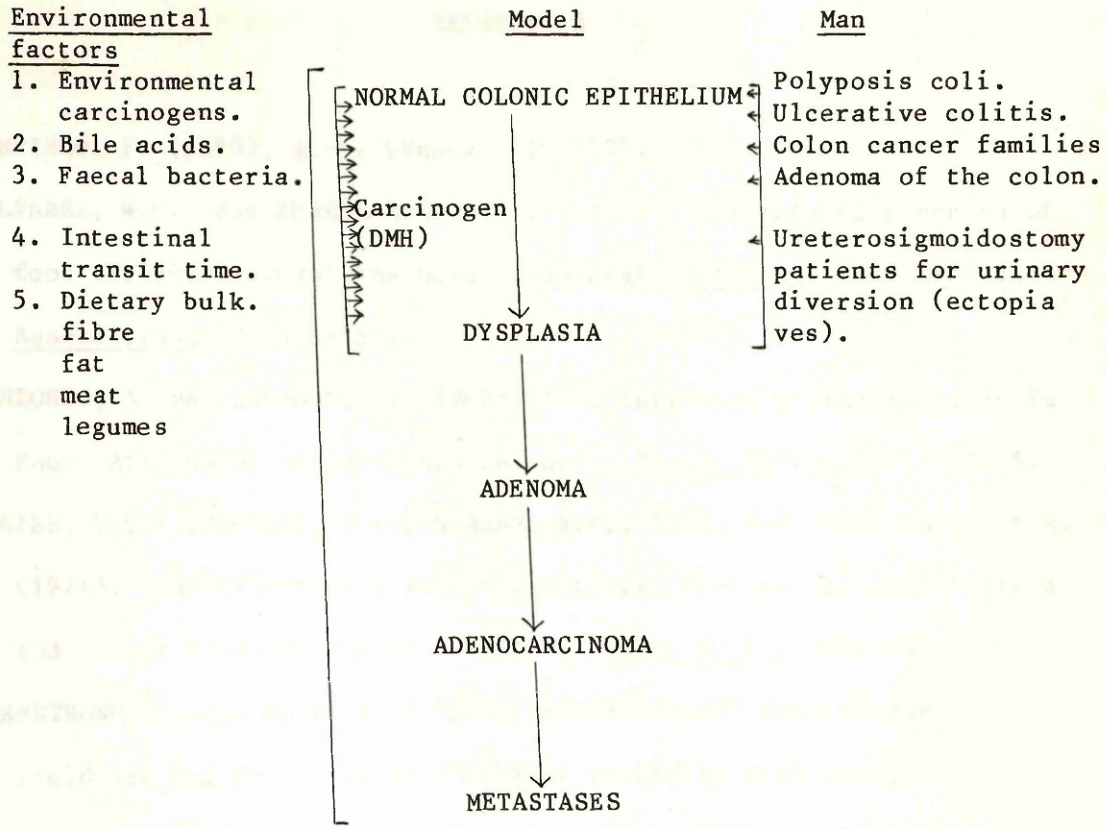


Illustration 8

HYPOTHESIS TESTED

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