THE EXPERIMENTAL PRODUCTION OF GASTRIC ADENOCARCINOMA

by

Robert M. Baird, M.D.

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Department of Experimental Surgery,
McGill University,
Montreal.

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PREFACE.

In the past, studies on factors affecting the experimental production of gastric adenocarcinoma have been hampered by the lack of a method which would produce adenocarcinomas of the stomach in a significant number of animals. Until 18 years ago there were no successful authentic cases of experimentally induced gastric adenocarcinoma. During the past 30 years many studies have been carried out, but the results of the best series have been at most disappointing.

The studies presented are the result of efforts to develop a satisfactory method of tumour induction. After a satisfactory method was developed, the effect of various modifying factors on the tumour induction was studied.

Dr. Stanley C. Skoryna has been interested in this subject for many years and to him is due much of the credit for the development of the method which is presented.

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PAGE

PAGE

PAGE

PAGE

TABLES AND FIGURES

I

INTRODUCTION.

The study of experimental gastric carcinogenesis of necessity must also involve a consideration of the problem of gastric carcinoma in the human. It is to be hoped that if the problem of human gastric carcinogenesis is studied carefully, methods of utilizing animals to elucidate these same problems may be found.

Cancer of the stomach is one of the most frequently occurring malignant tumours in humans. In Canada in 1955 there were 2,944 reported deaths from malignant neoplasms of the stomach (1). This constituted 14.3% of the total deaths from neoplasms and 34% of the neoplasms arising from the digestive organs and peritoneum. These figures include all the neoplasms of the stomach and not just the adenocarcinomas.

There is a marked age and sex variation in the disease. The older age groups and the males being more susceptible. Figures 1 and 2 illustrate these 2 variations as reported in Canada.

FIGURE 1.

Age-adjusted death rates for cancer of the stomach in Canada,

1941-1953.

Year.	Male	Female
1941	31.7	18.1
1942	30.9	17.9
1943	30.9	17.9
1944	29.9	16.8
1945	30.2	15.3
1946	28.2	16.8
1947	29.0	14.9
1948	27.9	16.1
1949	27.5	14.6
1950	26.6	14.3
1951	27.2	13.8
1952	28.0	14.0
1953	25.4	14.1

FIGURE 2.

Age specific death rate in Canada for cancer of the stomach in 1941 and 1953.

Age Group	1941		1953	
	Male	Female	Male	Female
20-29	1.0	0.7	0.4	0.6
30-34	1.4	0.7	2.0	2.3
35-39	7.1	3.6	3.8	3.1
40-44	10.3	5.8	9.9	4.8
45-49	22.9	12.2	18.7	11.3
50-54	43.8	22.1	35.4	13.2
55-59	76.8	41.5	59.5	24.0
60-64	114.1	62.6	83.8	45.0
65-69	177.6	115.1	126.2	75.7
70-74	272.1	127.5	203.1	112.6
75-79	362.1	223.4	320.8	169.9
80-84	385.3	272.7	397.6	224.9
85 -	295.6	212.1	324.9	253.2

These figures are of course based on mortality rates and it is of interest to compare them with incidence rates. In the province of Saskatchewan, cancer is a reportable disease and incidence rates are available (2). During the period 1932 - 1955, primary cancer of the stomach constituted 8.5% of the total reported cancers. There were other malignancies which were more frequent, such as cancer of the skin 21.3%, breast 11.3%, lip 9.8% and female genitalia 9.2%. Those malignancies with better prognosis tending to be proportionately higher in incidence.

The prognosis for this disease, which is usually expressed as five year survival rates, is very poor. Although the reports from various institutions with varying types of treatment differs, the overall five year survival does not exceed 20%. Barclay (2) has reviewed 12 reports on survival rates, the statistics varying from 3.5 - 14.0% five year survivals. In Saskatchewan (2) between 1932 - 1955 the 5 year survival rate in males was 5.2% and in females 5.6%.

The large majority of epithelial tumours of the stomach are

adenocarcinomas. The sites of origin with their frequencies are as follows (3).

Pylorus and antrum	47%
Lesser curvature	26%
Cardia region	10%
Remainder of organ	9%
Whole stomach	8%

Willis has classified carcinomas grossly and histologically as follows (3).

Gross Classification.

1. Polypoid or fungoid carcinomas of predominantly endogastric growth.
2. Ulcerated plateau-like carcinomas.
3. Ulcer-like carcinomas with diffuse infiltration of the neighbouring stomach walls.
4. Extensive diffuse carcinomas with only slight superficial ulceration or none at all.

Histologic Classification:

1. Adenocarcinoma or adeno-papillary carcinoma.
2. Mucoid adenocarcinoma.
3. Signet-ring cell carcinoma.
4. Infiltrating spheroidal-cell carcinoma.

5. Metaplastic squamous cell carcinoma.

6. Highly cellular anaplastic carcinoma.

The other types of tumours which may occur in the stomach include papillomas, adenomas, myomas, leiomyosarcomas, fibromas and fibrosarcomas, neurolemmomas, lymphosarcomas and lymphomas. It is the carcinomas which constitute the largest problem and will be the type studied in this paper.

II

ETIOLOGICAL FACTORS IN HUMAN GASTRIC CANCER.

As with many other diseases in which the pathogenesis and etiology are obscure, there are many apparently unrelated and poorly understood phenomenon known about gastric cancer. These unrelated facts are statistical in nature. As an example it has been demonstrated that the disease has a variable incidence in different racial groups. However the causation of this variability, as with the other statistical facts concerning the disease, could be due to many factors. The

causative factors could be exogenous ones such as dietary or climatic factors or endogenous ones such as familial or inherited metabolic factors.

Therefore the only possible means of study at present is the study of apparently unimportant facets of the disease, such as the blood group substances. However in the past the causation of diseases such as retrolental fibroplasia was elucidated by the examination of apparently unimportant environmental factors. Although the following observations do not give specific knowledge to the study of gastric cancer, they give many ideas on which to conjecture.

Racial Variation:

Snijders and Straub (4) in 1921 first drew attention to the marked discrepancy in incidence of gastric carcinoma between the native Javanese labourers and the Chinese labourers working together on the plantations in Java and Sumatra. Bonne (5) later elaborated on this study. Although the total incidence of all types of cancers is the same in the 2 groups, only 1% of the tumours in the Javanese were found to arise

from the stomach. In spite of this variation in incidence the causative factor remains unknown. Pathologically the tumours were similar in the 2 groups (6) and there was no difference found in gastric secretory components, pepsin levels and serum electrolytes.

Numerous other statistical studies have corroborated that there is a marked racial or national variation. The Japanese people have the highest incidence of any racial group. The standardized death rate in Japan for cancer of the stomach, compared with Canada, England and Wales for 1952 is as follows (7).

Country	Standardized Death Rate per 100,000	
	Males	Females
Japan	62.2	37.5
Canada	28.0	14.0
England and Wales	26.5	17.0

The disease in Japan follows the same sex and age patterns that it does in other countries (7). Steiner (8, 9) has examined the racial incidence of gastric carcinoma of Japanese living in Los Angeles; in his series 36.2% of all malignant tumours in

Japanese dying in the Los Angeles County Hospital were of gastric origin. This corresponds to the statistics reported from Japan (10). The Caucasoids in his series had only 12% of their neoplasms arising in the stomach. Grinspoon and Dunn (11) have demonstrated that the Japanese living in Los Angeles do not have an increased rate of achlorhydria over the Caucasoids. There are few first and second generation American-Japanese in the cancer age group as yet, therefore the effects of western diets and culture have not had an opportunity to alter the gastric carcinoma incidence rates.

The American negro has a higher incidence of gastric cancer than does the African negro (8, 12, 13, 14). This suggests that an ecological agent may, at least in part, be responsible for the variable race incidence. Doll (15) and Haenszel (16) have reviewed the statistics on this subject; it appears that the northern countries such as Finland, Iceland, Norway and the Netherlands have a proportionately higher incidence of the disease than do the southern countries.

The causative factors responsible for this racial variation

remain obscure. With the increasing intermingling and intermarriage of the worlds races it will be of interest to note if the racial incidence of gastric cancer is altered.

Familial Tendency:

An exaggeration of the increased familial incidence seen in the disease was manifested by the Bonaparte family. Napoleon himself died of gastric cancer (17, 18) as did his father, three sisters and a brother.

Graham and Lilienfeld (19) have recently written a critical review on genetics and gastric cancer. As they pointed out, it is very difficult to obtain accurate data on and design good experiments on this subject. Most of the studies have been based upon the determination of the incidence of the disease in relatives of patients with gastric cancer and the comparison with a control group. Videbaek and Mosbeck (20) with a study of this type, concluded that the incidence of gastric cancer is four times as great in cancer relatives as it is in the general population. Woolf (21) concluded that the incidence is twice that of the general population. Hogg (22) using different control

group produced ratio of 2.3, 2.5 and 2.1. However this increased tendency is not necessarily due to a genetic factor but may well be due to environmental factors. The genetic factor if present may be manifested by such means as the blood group substances.

Macklin (23), has made an attempt to isolate a common environmental factor, through investigating the incidence of gastric cancer in marriage partners as well as in relatives. Although his study revealed the usual familial tendency, there was no increased tendency to gastric cancer in marriage partners. This is only a single report, but it does suggest that environmental factors common to the household are not of great importance. Therefore factors such as diet, soils, trace elements, type of cooking utensils and methods of cooking, possibly have very little etiological significance with the disease.

Socioeconomic Differences:

It was first shown in Great Britain that the incidence of gastric carcinoma varies with the social class. The population of England and Wales was divided into 5 socioeconomic classes

and the incidence of gastric carcinoma in each group was estimated (24).

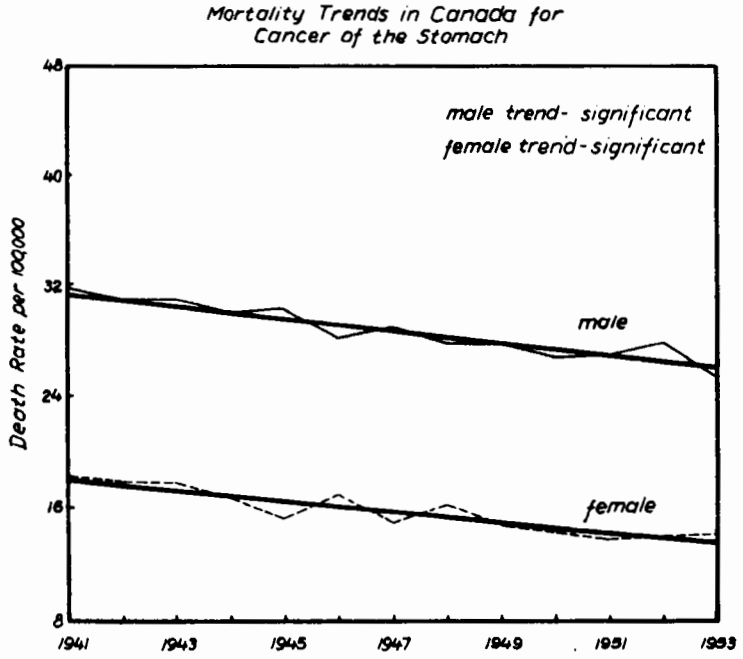
Social Class	Standardized Mortality Rate.	
	Men	Married women
1	57	57
2	67	72
3	100	101
4	114	106
5	132	138

The incidence has a definite tendency to increase in the lower income groups. Cohart (25) has shown that this phenomenon is true in the United States as well. Also certain racial groups on this continent, such as the negroes who tend to be in the lower classes, have a higher incidence of the disease than do the white people. This socioeconomic variation, perhaps more than the racial variation, suggests that environmental factors, such as dietary deficiencies, are related to the genesis of gastric carcinoma in the human.

Changing Trends in Mortality Rates:

Another statistical finding, the causation of which may be the same as the altered social class incidence rates, is seen when the corrected mortality rates are compared for the past 20 to 30 years. In Canada the age-adjusted mortality rate for cancer of the stomach in males has dropped from 31.7 in 1941 to 25.4 in 1953 (26). Graphically this appears as follows (26).

FIGURE III.



This trend has also been demonstrated in the following countries; (15, 16, 27)

Death Rate from Cancer of the Stomach per 100,000 males.

Year	Country			
	England & Wales	Holland	Norway	U.S.A. (whites)
1928	96			
1930			213	88
1939		167		
1951	91	116		
1952			150	48
1954	84			

There is as yet no known basis for this decrease in the mortality rates. It possibly is due to the same factor or factors which cause the variable incidence with economic class, as so many of the recent changes in our society, such as improvements in sanitation, control of contagious diseases, and improved nourishment, are the advantages which the upper classes have enjoyed over the lower classes for so many years.

Although the operative mortality rate for gastric resection has decreased considerably in the past 40 years, the actual 5 year survival rates (2) have not been altered significantly. Therefore this change in incidence must be due to an actual decrease in incidence of the disease, not just an improvement in methods of treatment.

The comparison of incidence and mortality rates in Saskatchewan indicates this also (28).

Blood Group Substances:

Aird (29) in 1953 first reported that there was a statistical relationship between the ABO blood groups and cancer of the stomach. This has since been confirmed by reports from all over the world (30, 31, 32, 33, 34, 35, 36). Hogg and Pack (34) have compiled the results of 6 of these studies and added one of their own. After statistically analyzing the results, they concluded that in 5 of the 7 reports the results are statistically significant, and although not significant in the other 2 there is a similar trend. The overall average of "differences

in percentage in group A for gastric cancer from the normal population" was 7.12% in the 5 significant studies.

Patients in group A have an increased tendency to gastric cancer, those of group O have a decreased tendency and group B and AB have no significant tendency. The pathogenesis of this phenomenon has not been clarified as yet. Possibly it is a statistical error due to stratification of the population or possibly it is simply a matter of genetics.

The blood group substances themselves may be involved. Chemically the blood group substances are mucopolysaccharides (37, 38, 39) which are found throughout the body. The substances of the ABO and Le groups are similar chemically and only differ in their antigenic properties;their role in the body metabolism has not been determined (37, 38). In approximately 80% of the population the blood group substances of the ABO system are secreted in the secretions of the alimentary tract. It has been postulated that these substances either protect or else make the stomach more susceptible to malignant degeneration. Aird (40) has suggested "The mucopolysaccharides in the stomach exercise a protective action, blood

group O protecting particularly against carcinogenic influence and blood group A protecting particularly against ulcerogenic influence".

Other gastrointestinal diseases such as peptic ulceration (41, 42, 43, 44, 45) and pernicious anaemia (42, 44, 45) have also been shown to have a relationship to blood group substances. However apart from an isolated report (46) of an association between cancer of the uterus and blood group O, there does not appear to be any association apart from that with gastric cancer, between other malignant tumours and the ABO blood group substances (47, 48, 49).

Although the blood group secretor status of peptic ulcer patients has been investigated (41, 42, 43, 45), no similar studies have been reported on gastric cancer. The protective effect, if any of the blood group substances, does not appear to be a simple physical or chemical effect but may involve antigenic factors. Estimations of the actual concentration of the blood group substances in saliva has been done. Since the blood group substances are the only substances in alimentory secretions which contain fucose (50), it is possible to analyze for fucose

levels in saliva and from these values determine the concentration of blood group substances. However since all the ABO, H. and Lewis substances are similar chemically, the fucose test will not differentiate between the groups. But estimation of fucose concentrations in the saliva of normals and those with duodenal ulcers has shown that the ABO nonsecretors have similar fucose levels to the secretors (51). Therefore in duodenal ulceration at least the effect of the blood group substances does not appear to be a direct chemical or physical effect.

Environmental Factors:

Factors which have been considered, such as the variation in racial incidence and the variation in incidence with the social class, suggest that environmental factors may possibly affect gastric carcinogenesis. However as yet it has not been possible to relate significantly any environmental factors to gastric carcinogenesis. Of the factors studied the observed racial and socioeconomic variations have stimulated much work on dietary factors. The following substances have been

studied: green vegetables (52), alcohol and beer (24, 52), hot food (53), and diets excluding meat (54). None of these substances appear to be related to gastric carcinoma.

Stocks (52) in Great Britain has undertaken an extensive statistical survey of environmental factors and their effect on the cancer rate in various organs of the body. His only positive correlations regarding gastric carcinoma were of a minor degree and were for such substances as fried foods, certain trace elements in the soil and certain types of rock formations adjacent to the homes of patients. Tromp and Diehl (55) after studies on the soils in the Netherlands concluded that there is an increased tendency to gastric cancer in those living on peaty soils over those living on sandy or river clay soils. These associations are of minor nature only.

Other factors such as smoking, appear to be unrelated (15, 52). Kraus et al (56) reported that metal product workers are somewhat more susceptible to gastric cancer, however apart from this isolated report no other occupations have been

incriminated (52).

Recently attention has been directed to the possible presence of known carcinogenic hydrocarbonsin foodstuffs.

Such foodstuffs as coffee (57), smoked fish (58), charred dough,as occurs with excessive baking or toasting (59) and the paraffin wax used to coat food containers have been shown (60) to contain small quantities of known carcinogens. Again the significance of this is not evident as yet. There is no evidence that chemical carcinogens are a significant factor in human gastric carcinogenesis, although the induction of tumours by such means ought to be possible.

Precancerous Conditions:

Certain conditions such as gastric polyps, gastric peptic ulcers, gastric atrophy and pernicious anaemia have been suggested as being precancerous. Polyps or papillomas of the stomach are in themselves benign, but they do appear capable of undergoing malignant degeneration. The single and multiple polypi must be differentiated from polypoid or hypertrophic gastritis, for the latter is purely inflammatory and not

premalignant (61, 62). Stewart (63) found carcinoma in 27% of 56 stomachs with polyps, Cromer et al (64) 40% and Kettunen (65) 28%. Since gastric polyps are rare, occurring in less than 5% of the population (65), they are not a common cause of gastric carcinoma, only about 5% (63) of carcinomas originating from them.

Much has been written concerning the malignant potential of benign gastric ulcers. The problem can be studied from both the clinical and pathological point of view. Clinically Brown et al (66) reported that in the Cleveland Clinic from 1945 - 1951 only 1.1% of the benign ulcers developed into malignancies. The problem here of course is in what proof the author has for the benign state of the ulcers, short of direct biopsy a malignant tumour may be misdiagnosed as benign. Balfour (67) followed 1280 patients who had had gastroenterostomies for gastric ulcer. In these patients the gastric ulcer was usually not excised, however only 6% of these patients died of gastric carcinoma. From a pathological standpoint, carcinoma can be examined histologically and

evidence that the tumours were preceded by ulcers assessed. Stewart (63) found 16% of gastric carcinomas which he considered to show evidence of previous ulcers. Newcomb (68) found 13% and Dible (69) only 6%.

Certainly the problem has not been definitely elucidated as yet. Willis (3) states "Chronic ulcer must be regarded as only an occasional precurser of cancer", and Boyd (70) states "The present consensus of opinion is that not more than 5% of simple ulcers become malignant so that it is a comparatively rare occurrence, and that 10 - 15% is the maximum figure for cancers arising from such ulcers".

Although it would not appear that gastric ulcers are an important cause of adenocarcinomas, the appearance from time to time of a stomach with a gastric ulcer thought to be benign, which has the incidental finding of a focus of malignant cells in the base of the ulcer, indicates that carcinomas can arise in gastric ulcers on rare occasions. There is no evidence however that the peptic ulceration was carcinogenic in any way.

In 1929 Hurst (71) first suggested that the achlorhydria frequently associated with gastric carcinoma was not the result of the tumour but had preceded it. Since this time there has been much controversy regarding the relationship between gastric atrophy, achlorhydria and gastric carcinoma. Certainly gastric carcinoma is frequently associated with the condition, for in the normal population achlorhydria or hypochlorhydria (acid less than 30^{o}) occurs in approximately 25 - 35% of the normal population (72, 73), while 55 - 80% of those with gastric cancer are hypochlorhydric (72, 74, 75, 76, 77, 78).

As regards the significance of the achlorhydria, in racial groups such as the Javanese and Chinese who have contrasting incidences of the disease (6), no significant difference has been found between them with gastric acid and pepsin determinations. The Japanese in the United States also do not have an excessive incidence of achlorhydria (11). Comfort et al (75) investigated 277 cases of gastric carcinoma who had had gastrointestinal investigations prior to the development

of their disease. Of these 277 cases only 45% were achlorhydric 2 or more years before the tumour was diagnosed. However in a more recent article (79) in 1953, Berkson and Comfort after following 850 cases of achlorhydria for 10 years state "no convincing evidence was found of a larger incidence of development of cancer in this group than expected in the general population". In this latter article no statistics to support this statement are given.

Pernicious anaemia which is characterized by gastric atrophy has a controverseal relationship to gastric carcinoma. Many series with variable results have been reported on this relationship. Kaplan and Rigler (80) reported 293 necropsies on patients with pernicious anaemia, 12.3% of these have carcinoma of the stomach an incidence which is three times that of a comparable normal age group. Zamcheck et al (81) with their pernicious anaemia cases at the Boston City Hospital concluded that 10% will eventually develop gastric carcinoma. Videbaek and Mosbech (20)

estimate that pernicious anaemia patients are three times as prone to gastric malignancy as the general population. Berkam (82) at the Mayo clinic and Cotte (83) report similar findings.

Of those who disagree with these conclusions, Wilkinson (84) followed 1820 pernicious anaemia cases for a period of 21 years the number of gastric carcinomas occurring in this group was no greater than in the general population. Willis (3) states "In my opinion, the evidence that true pernicious anaemia or achlorhydria predisposes to carcinoma of the stomach is inconclusive". However it is difficult to reconcile the results of series with a positive correlation and Willis's statement. Although the mechanism of the relationship is obscure there does seem to be a definite relationship between gastric atrophy, with or without pernicious anaemia and gastric carcinoma.

The relation of gastric carcinoma to chronic gastritis is complicated by the confusion in the meaning of the term "gastritis". It would appear that to most authors it is

synonymous with gastric atrophy. Since gastric atrophy is associated in most cases with achlorhydria, the association between gastric cancer and gastric atrophy is similar to that between achlorhydria and gastric cancer. Hebbel (85) uses the term gastritis in a restricated sense to designate a series of changes manifested chiefly in the mucosa of the stomach. Histologically there is an excessive accumulation of lymphocytes and plasma cells, and atrophy and abnormal regeneration of the glands. Konjetzny (86) first suggested that these gastric mucosal changes were precancerous. However, others (87) have shown that these same changes are present in a high proporation of stomachs free of ulcer or carcinoma.

Hebbel (85, 88) studied stomachs from autopsy material in which the subjects had had no gastric complaints and also surgically resected specimens from patients with ulcers and carcinomas. He concluded that the mucosal changes described as "chronic gastritis" were present in 30% of normal stomachs in those over 50 years of age, present in all those stomachs with gastric ulcers, rare in stomachs with duodenal

ulcers and present in most of the gastric carcinoma specimens. However the microscopic findings suggested that the mucosal changes were secondary to the tumour. Therefore chronic gastritis, which is a variant of gastric atrophy, does not appear to be precancerous to a significant degree.

Intestinal metaplasia of the gastric mucosa has been proposed by Morson (89) as a precurser of gastric carcinoma. He believes that (90) "the presence of intestinal epithelium in the stomach is the result of faulty regeneration of surface epithelium in a mucosa repeatedly damaged by gastritis and is an example of metaplasia resulting from chronic irritation". He concluded (89) that in 35 of 107 (32.7%) gastrectomy specimens for carcinoma, the tumour appeared to be arising in epithelium of intestinal type. Jarvi and Lauren (91) and Berg (92) also believe that intestinal metaplasia is of importance in gastric carcinogenesis. However (93) irritation of the gastrointestinal tract will produce excessive mucous production and an increase in the mucous secreting cells, which are the changes that Morson describes as "intestinalization". Therefore

Morsons intestinalization is probably the result of irritation caused by the tumour and not the tumour a result of it.

Biochemical Alterations:

There are very few specific systemic biochemical alterations in gastric carcinoma. As with other types of malignancies the serum proteins are not altered specifically (94, 95, 96) although Glass (97) has noted changes in the mucoproteins of gastric juice. There are no specific hormonal changes (98). It has been noted by Changus and Dunlop (99) that the gastric juice from cases of gastric carcinoma contains abnormally high levels of acid phosphatase. The significance of this is not evident at present.

Japanese workers (100) have reported the presence of a substance with a specific biologic activity (K.I.K. factor) in the gastric juice of gastric cancer cases. This substance on intravenous injection in rabbits will lower the animals erythrocyte count. Whether or not this substance is restricted to gastric carcinoma remains controversial and doubtful at the present time.

Spontaneous Regression:

Gastric adenocarcinoma, as with many other types of tumours, has the ability on occasion to undergo spontaneous regression. The phenomenon of spontaneous regression has been observed for many years, Rohdenburg (101) reviewing the subject in 1918. Konjetzny (102) reported a case of gastric adenocarcinoma and Rohdenburg (101) referred to one of Schuchardt's. Everson & Cole (103) in 1956 reviewed the subject and were only able to find one case which fulfilled their requirements of acceptability, although including other tumour types, 47 cases in all were found.

This rare and strange phenomenon suggests that there has been an alteration in the cellular environment or else a withdrawal of an etiological factor. Withdrawal of an unknown carcinogenic agent from a carcinogenic dependent tumour could cause the tumour to regress. Other factors which have been suggested (103, 104) include alterations in endocrine environment, alteration in the nutrition of the tumour or possible a partial excision of the lesion results in the removal of a key part of the tumour which then regresses.

III

EXPERIMENTAL GASTRIC CARCINOMA.

Spontaneous gastric Tumours in Animals:

Although gastric carcinoma is a very frequently occurring malignancy in humans it is a very rare disease in animals. The following reported series indicate the extreme rarity with which it is seen. Wells (105) autopsied 142,000 mice of the Slye stock dying of natural causes and found only 3 cases of gastric adenocarcinoma. Wooley (106) autopsied 23,000 wild rats and found only 23 tumours, none of which were gastric adenocarcinomas. Crain (107) found only 2 gastric adenocarcinomas in 200 spontaneous tumours in the wistar rat and Ratcliffe (108) also with the wistar rat found none in 273 spontaneous tumours. Bullock (109) with postmortems on 33,000 wild rats found 1 gastric adenocarcinoma and McCoy (110) found none in 100,000 wild rats. As well as the rodents, it has been shown to be a rare disease in domestic, laboratory

and wild animals, fish, reptiles and insects (111, 112). Ruddick and Willis (113) describe an adenocarcinoma of the cardia in a dog and go on to state that the stomach is one of the rarest sites of cancer in the dog. Ratcliffe (111) autopsied 3400 mammals at the Philadelphia Zoological Gardens. He found 96 tumours three being adenocarcinomas of the stomach, one in a baboon and two in kangaroos.

Recently two possible exceptions to this phenomenon have been reported. Fortner (114) observed 301 Syrian hamsters for a period of age to 2 years during which time he recorded the occurrence of 14 primary adenocarcinomas of the glandular stomach, small and large intestine. However he does not state specifically how many tumours arose in the stomach, he does not include photographs of the lesions and he does not state his criteria of malignancy.

A colony of Syrian Hamsters have been kept in the McGill laboratory for the past 2 years. During this period there have been no spontaneous gastric tumours observed. Until Fortners work can be confirmed there does not appear to be

sufficient evidence to consider the hamster susceptible to spontaneous gastric adenocarcinoma.

Oettle (115) has reported that 40% of the deaths in his laboratory colony of mastomys were from spontaneous adenocarcinoma of the stomach. The mastomy or Rattus natalensis is a small, brown, grain eating rodent native to South Africa. In a subsequent publication (116) he suggests that an extrinsic factor such as a parasite may be responsible for these lesions. Other experimenters (117) have noted spontaneous gastric adenocarcinomas in this animal, although not in as high a percentage as did Oettle.

Criteria of Malignancy:

In a consideration of experimental tumourogenesis it is important to define what constitutes a malignant versus a benign tumour. The assessment of human tumours, although at times difficult, is perhaps easier than it is with animals. For in humans the pathologists have had much more experience with the clinical result of the various histologic types, and have had more experience upon which to base their diagnosis.

Also in the human a suspect lesion may be treated as a malignant lesion when the histological diagnosis is in doubt. In animals however it is necessary to state arbitrarily which are malignant and which benign. For this reason, in experimental tumour work more objective criteria of malignancy have been proposed. Klein and Palmer (118) however in their excellent review state that it is impossible to be completely objective in the assessment of tumours. They suggest that induced tumours should have the following characteristics to be considered malignant:

1. Ability to proliferate independently as metastases.
2. Ability to invade progressively and destructively.
3. Irreversibility of these properties in the absence of the entrinsic factor initially held responsible for the cellular changes.
4. Reasonable evidence to indicate a causal relation of the experimental procedure to the tumour.

It is their opinion that the histologic appearance of the tumour alone is not an adequate basis for the diagnosis of malignancy. Others do not agree with them on this point.

Stewart (119) in his extensive studies on induced gastric tumours appears to rely on histologic appearance and he does not consider metastases as prerequisites. However he lays much stress on the invasive properties of a tumour. He believes that a tumour must actually have invaded the serosa to be considered malignant. Consequently a lesion which is histologically malignant but which has not invaded the serosa he will classify as benign.

Rodent tumours seldom independently metastasize although invasive powers are exhibited, therefore the first criteria of Kleins would appear to be unnecessary. Also other malignant characteristics such as anaplasia and invasion can occur in benign tumours. It is important to consider in each tumour the sum of the malignant characteristics which it possesses, for one characteristic alone is not enough to classify the tumour as malignant.

Dietary Carcinogenesis:

Various deficiency diets will give rise to benign lesions of the stomach although there are no valid reports of malignant

lesions. Because of the reports of Fibiger (120) and Roffo (121), vitamin A has been studied extensively. A vitamin A deficient diet will give rise to papillomas of both the forestomach and glandular stomach. Howes and Vivier (122) believe that these lesions are not due primarily to the vitamin A deficiency but to the anorexia caused by the special diet with subsequent multiple deficiencies. However they have not demonstrated any evidence for these multiple deficiencies and other observers (123, 124) have not reported a loss of appetite in their animals on the deficient diet.

Vitamin B_2 deficiency will also cause a papilliferous hyperplasia of the glandular stomach (123). A low protein diet will cause ulceration of the glandular stomach (122) and a low choline diet causes papilloma formation.

The carcinogenic effect of ingested substances which are physically irritating to the stomach has been investigated. Salmon and Copeland (125) fed tributyrin to rats, which resulted in papillomatous growths of the forestomach and in the glandular stomach; cysts lined with glandular epithelium

or lesions of proliferating gland elements deeply placed in the submucosa and in the external muscle layer.

The irritation appears to cause a hyperplasia of the gastric mucosa, the cysts are due to an enclosure of mucous or secreting cells so that their secretions accumulate in the cystic space. The glands hypertrophy appear to invade the muscle, although they are benign.

Hair ingested by rats gives rise to inflammatory changes in the stomach (126). This may progress to hyperplasia with glands apparently carried into the submucosa and muscularis, but no evidence of neoplasia.

Parasites:

Fibiger (120) in 1913 reported that he had induced squamous cell carcinomas of the forestomach in rats by infesting them with the nematode, Gonglylonema neoplasticum. As this was the first report of a successfully induced malignant tumour, the study gained much recognition and Fibiger was subsequently awarded the Nobel prize for his work. Since this time there

has been much controversy regarding this report, a number of investigators presenting evidence that his tumours were not malignant or due entirely to the parasite (118, 124, 127, 128). The present opinion is that the lesions produced were simply an epithelial overgrowth and not a true neoplasm. Also at the time of his publication there was very little known about nutrition. Fibiger fed his animals exclusively on white bread and water which is a diet deficient in vitamin A. It has been shown that animals fed a vitamin A deficient diet and with no parasitic infestations will develop gastric lesions very similar to those described by Fibiger (123, 124). Also animals infested with the parasite and fed a balanced diet will develop only minor gastric lesions (124).

Other parasites have also been implicated in gastric tumourogenesis. Blumberg and Gardner (129) infested rats with Cysticercus fasciolaris, which is the larval form of the tapeworm Taenia taenialformis which infects cats. In the rat the larvaeencyst in the liver. The authors note that when there was a heavy infestation in the liver, the glandular portion

of the stomach developed polyps, adenomatous changes and abnormal glands. These changes occurred when the stomach was free of worms and presumably is due to a dietary deficiency or to a toxic substance.

Oettle et al (116) have noted that infestations with Bilharzia mansoni in the mastomy does not affect the rate of spontaneous gastric carcinomas, although it does give rise to hepatomas.

Chemical Carcinogens:

The carcinogenic effects of certain chemicals was first observed many years ago. In 1775 Percival Pott described chimney-sweeps cancer of the scrotum, which was the first clearly recognized manifestation of occupational cancer. Successful experimental carcinogenesis began with the work of Yamagiwa and Ichikawa (130) in 1918. At this time they first described the induction of carcinoma in a rabbits ear following the repeated painting of it with coal tar. Subsequent studies showed that the various tars each had different

carcinogenic effects which suggested that specific substances were responsible for the carcinogenic effect.

Other groups of chemical compounds including the azo compounds and the aniline dyes have been shown to have carcinogenic qualities. Hartwell (131) has compiled a list of the compounds tested for carcinogenic activity, at present 322 chemicals have been proven to be carcinogenic.

Oral Carcinogenesis:

The induction of gastric adenocarcinomas in animals with chemical carcinogens has been attempted by several methods. Because of the stomachs role in digestion, the oral administration of carcinogens has been the route most extensively studied. Many workers over the years have tried many different substances in an effort to induce carcinomas of the glandular stomach. It was hoped by many that an oral gastric carcinogen could be found. However in spite of many extensive studies, no chemical has been found which will induce adenocarcinoma of the glandular stomach in rats or mice. Benign changes often occur but not true neoplasms. The

following is a summary of some of the substances used orally and their effects.

Polycyclic Hydrocarbons.

1. 3:4 - Benzypyrene : Will produce squamous cell carcinomas of the forestomach but no true tumours of the glandular stomach (132, 133, 134).

2. 1:2:5:6 - Dibenzanthracene : Produces papillomas of the forestomach, adenocarcinomas of the small intestine and multiple primary tumours of the lung (132, 133, 135).

3. 9:10 - Dimethyl - 1:2 - Benzanthracene : Produces squamous cell carcinomas of the forestomach (133, 136, 137, 138).

4. 20-Methylcholanthrene : Most frequently used substance, produces squamous cell carcinomas of the forestomach, adenocarcinomas of the colon and rectosigmoid (133, 135, 139, 140, 141, 142, 143, 144, 145).

Azo Compounds.

1. 4-Oxyazobenzene. Produces benign papillomas of the stomach in rats (146).

2. P-Dimethylaminobenzene - 1 - Azo - 1 - Naphthalene : Produces squamous cell carcinomas of the forestomach (147).

3. 3:4:5:6 - Dibenzcarbazole : Does not produce lesions of the glandular stomach (148).

4. P-Aminoazobenzene : Does not produce any gastric lesions (149).

Aromatic Aminos.

1. 2-Acetylamino-fluorene : Tumours of the forestomach, thyroid, renal pelvis, female generative tract and breast, bladder and liver (150).

Others.

1. Urethane (ethyl carbamate) Papillomas of the forestomach (151).

Although the glandular stomach is resistant to oral carcinogens, it is possible (152, 153) to induce gastric adenocarcinomas, if the deeper tissues are exposed to the carcinogen. This phenomenon suggests a mucosal barrier of some type, perhaps the same barrier that has been postulated to explain the lack of autodigestion of the stomach. Experimental attempts have been made to destroy this barrier and then feed carcinogens.

Aqueous soap solutions which can dissolve mucous have been fed with carcinogens (132). Also lipo and hydrophilic

substances in the form of association colloids have been utilized (138). These substances were followed with fluorescein methods and it was observed that there was some absorption of the carcinogen into the stomach wall, although no malignancies were produced. Polyethylene glycol-400 is also a lipophilic-hydrophilic solvent and appears to have no effect on gastric carcinogenesis (133, 137). Tween 80 is a surface active agent which is a wetting agent and emulsifier. Although it did not affect the glandular tumour induction, when fed with 3-methylcholanthrene, the incidence and severity of tumours in the forestomach was increased and the incidence of tumours arising distant from the gastrointestinal tract was increased (139). Bile does not have any carcinogen potentiating effect (140).

Gastric irritants such as eugenol which causes the desquamation of gastric mucosa is also ineffectual (141). Hitchcock (141) heated methylcholanthrene to 65° C. and fed it via gastric tube in mice, the carcinogenic effects of the methylcholanthrene were not altered. Deficiency diets which as has been mentioned will give rise to benign changes, have

been combined with carcinogens (145). Diets deficient in vitamin A, vitamin B complex and protein were combined with methylcholanthrene. The induction period of the fore-stomach tumours was shortened but the actual tumour incidence was not altered.

Attempts have been made to destroy the normal mucosa by creating excision ulcers (142, 143). These studies were done in rabbits for this animal is very slow to heal gastric ulcers. The ulcers did not alter the effect of the oral methylcholanthrene. Ray et al (154) attempted to circumvent the mucosal barrier by causing secretion of the carcinogens by the gastric mucosa. Dyes were found which could be shown to be secreted by the stomach; these were then conjugated with carcinogens. All the lesions produced were of a benign character and included gastritis, erosions, ulcerations and papillomas.

All of these attempts at destroying or bypassing the mucosal barrier have been unsuccessful. The nature of this "mucosal barrier" remains theoretical.

Perhaps the carcinogen passes through the stomach so quickly that it is not exposed to the gastric mucosa for a sufficient period of time. Kobernick and Toovey (155) attempted to disprove this theory. They exteriorized gastric mucosa in the rat and then repeatedly painted it with carcinogens, No malignancies developed. However the mucosa was found to secrete large volumes of fluid, therefore the carcinogen may have been washed off very rapidly.

The gastric mucosa has been shown to have a very rapid replacement of the superficial cells. (156). Stevens and Leblond (157) estimate that the surface epithelial cells are replaced every 3 days and the mucous neck cells every 7 days. Therefore it is possible that the cells exposed to the carcinogen are discarded before malignant degeneration can occur.

Although mucous solvents have been unsuccessful at removing the carcinogenic barrier this does not negate the mucous barrier theory. For if the solvent is strong enough to remove all the mucous it might also kill the exposed cells. Also the gastric glands are deep and narrow and relatively

inaccessible to both the solvent and the carcinogen. It is at the bottom of these glands that the immature susceptible cells lie and it is quite possible that the carcinogen does not come into contact with them.

Intramural Injection of Carcinogens.

Although the glandular stomach is resistant to carcinogens exposed to the mucosal surface it is susceptible to chemical carcinogenesis. Adenocarcinomas of the glandular stomach will develop if the carcinogen is placed in the submucosa of the stomach. Stewart and his co-workers were the first to demonstrate this and have done most of the subsequent work, although their results have been conferred by others (158). The carcinogens used have been exclusively hydrocarbons, in particular 20-methylcholanthrene and 3:4 benzypyrene. The solvents for the carcinogens include water, (158, 159) sesame oil (160) methocel (161, 162) liquid petrolatum (163), horse serum (153, 164) and corn oil (165). The animals used, rats and mice. The method involves exposing the stomach by

laparotomy and then injecting the substance into the submucosa along the greater curvature. Various sites on the greater curvature have been utilized.

The results with this method in mice have been variable. The incidence of gastric adenocarcinomas varying from 0 to 15% (158, 159, 160, 161, 162, 163, 164 166). The types of tumours produced include adenocarcinomas, adenoacanthomas, squamous cell carcinomas and combinations of these (161). It is difficult to evaluate the effect of the various solvents used, since the various studies were done on different strains of animals, different dosages were used, different sites of injection utilized and the animals kept for variable periods.

The site of injection affects the types of tumours produced. Injection of the substance into the forestomach will produce a preponderance of squamous cell carcinomas and no adenocarcinomas (159). Injection in the fundus adjacent to the limiting ridge produces more sarcomas than does injection near the pylorus (167).

Stewart et al (161, 166) have attempted to evaluate the effect of the strain of the mice. They concluded that strains C, C57BL, C3H, C3Hb, were approximately equally susceptible. The C57BL mice were somewhat less susceptible to the induction of sarcomas. Mice of strain DBA were resistant to induction of adenocarcinomas.

The rat has not been used as extensively as the mouse in this work although the results are comparable. Rusch et al (165) in one of the first attempts utilized 3:4 - benzypyrene and reported a tumour incidence of 20% . However his results have been critisized by others (165, 167), who feel that the lesions were only precancerous adenomatous diverticulae.

The strains of rats used have included Osborne-Mendd, Marshall 520, Ax C9935, (24) with little strain difference noted. Again the types of tumours are similar to those in the mouse, the rats tending to demonstrate adenomatous diverticulae which are not seen in the mouse (168). The tumour induction period is from 12 weeks (167).

Submucosal Threads:

The other method of exposing the submucosa to the carcinogen is to insert a thread saturated with the carcinogen in the submucosa. Threads saturated with 20-methylcholanthrene, 3:4 - benzypyrene and dibenzanthracene have been used. There have only been three studies of this type reported (152, 169, 170, 171). Stewart (169) in 1943 first used Andervonts (172) technique and implanted threads in the submucosa of the stomach. Their study was only on a small group of mice and the only malignancy produced was a sarcoma. Howes and de Oliveira (152) subsequently implanted threads in the rat. Their interest seems to have been chiefly concerning the various histological responses to the thread and they do not state how many tumours were produced. Grant and Ivy (170) and Ivy (171) have published 2 papers on this subject presumably using the same group of animals. No adenocarcinomas of the stomach were produced although 3 adenoacanthomas were produced (170). In this study the effects of concurrent mucosal injury was followed. The bed of the thread being cauterized in some animals and in others the muscularis propria

was excised. This did not affect the tumour induction rate as no adenocarcinomas occurred in any case. The animals were only kept for 1 year, this is probably why more tumours did not occur.

Howes and de Oliveira (152) removed the thread at periodical intervals in some animals. They found that if the thread was removed on the 35th day no tumour resulted. However if the thread was not removed until the 60th day, then tumourogenesis occurred. In animals in which the thread was not disturbed they found that it was usually gone by 150 days.

Of the 2 methods of bypassing the mucosal barrier that of injection of the carcinogen has been more extensively studied. Of those studies utilizing the thread method, none of them have been carried out for periods over a year and the experimentors were apparently chiefly interested in following the chronologic histologic changes.

Cancer Susceptible Strains:

Strong (160, 173, 174, 175) has experimented with gastric cancer susceptible strains of mice and reports that he has developed a strain which develops spontaneous gastric adenocarcinoma. By taking mice of the NHO strain and injecting subcutaneous methylcholanthrene in sesame oil he found 5 adenocarcinomas of the stomach in 2000 mice (160). He then proceeded to inbreed the tumour susceptible mice and subsequently developed a strain with a gastric tumour incidence of 66.2% (173). At this time he reported a strain developed which spontaneously developed gastric lesions without the subcutaneous methylcholanthrene. His tumours have been examined by independent observers (117, 126) and it is their opinion that the gastric lesions are only adenomatous lesions and not true neoplasms. There has been no other reports of gastric cancer susceptible strains of rodents.

Radiation:

It has not been possible to induce carcinoma of the glandular stomach in a significant number of animals with ionizing

radiation. The only positive report concerned the use of a whole body dose of fast neutrons (176, 177), these authors report that 4 adenocarcinomas arose in the glandular stomachs of 162 mice and, that is in 2%. In this series 5 squamous cell carcinomas occurred and 58 cases of nodular hyperplasia of the stomach. Another study in which a single total body dose of X-irradiation produced only 1 adenoacanthoma in 192 mice (176, 177). Radiation directed to the stomach by means of a gastric balloon containing Phosphorus 32, produced no tumours of the glandular stomach although squamous cell carcinomas did occur (178). Saxen using irradiation directed to the stomach with doses of up to 1000 R did not report any adenocarcinomas of the stomach (136). He also combined irradiation with oral carcinogens and was unable to produce glandular tumours. Kaplan irradiated N.H.O. strain mice with 1000 R of whole body roentgen irradiation and simultaneously injected subcutaneous methylcholanthrene (117). Neither of these stimuli had any effect on the incidence of adenomatous lesions in the stomach. The human stomach also does not appear to be susceptible to irradiation for the survivors of the atomic blasts in Japan (179)

and those exposed to roentgen irradiation (180) have not shown an increased incidence of gastric carcinoma.

Transplantation of Tumours:

Few successful transplants of the induced gastric carcinomas have been reported. Stewart et al (161) using the tumours induced with injected methylcholanthrene, attempted transplants in 50 mice. Successful transplants occurred with the following tumour types:

Adenocarcinomas :	1 successful transplant
Adenoacanthomas :	4 successful transplants which grew as pure squamous cell carcinomas.
Squamous cell carcinoma:	1 successful transplant
Sarcomas:	15 successful transplants.

Oettle (115) in his report of spontaneous tumours in the mastomy, reports that homologous and heterologous transplants were unsuccessful apart from an homologous brain implant.

Effect of Heated Fats:

Cholesterol and other fats are very similar chemically to

the cholanthrene (181). Consequently much attention has been directed to cholesterol as a possible endogenous carcinogen. Roffo (121, 182) has attempted to show that heating fats and cholesterol such as might occur during deep frying converts the fats into carcinogens. He fed rats preheated fats and reported the induction of gastric adenocarcinomas. Subsequent reports from other workers (123, 171, 183, 184, 185) state that the lesions are in reality not true neoplasms. Peacock (184) who repeated Roffo's work considers the gastric lesions due to a vitamin A deficiency, the heating of the fat destroying the fat soluble vitamins. Kirby fed the same preheated cholesterol but then added supplemental vitamin A and reported that the rats did not develop gastric lesions (123).

IV.

MATERIALS & METHODS.

Object.

The review of the literature has shown that there is no method reported which will produce adenocarcinoma of the glandular stomach in a significant number of animals. In this paper several methods of tumour induction are presented and their results discussed. The effect of various diets and administered drugs on tumour induction has also been studied.

Experimental Animal:

The animals utilized in this experiment were male rats of the Royal Victoria Hosptial strain. These hooded, or piebald rats are the offspring of Wistar and Norway wild rats. The colony has been highly inbred from brother and sister matings since 1933. During these years there have been no cases of spontaneous gastric adenocarcinoma noted. The animals are not prone to any particular type of tumour, the chief cause of death being pulmonary infection.

The animals were kept in stainless steel cages of the hanging type with 0.5 inch wire mesh bottoms for the passage of urine and faeces. Up to four animals were kept in each cage, depending upon the size of the animals. Room temperature was maintained between 70 - 78° F. and ventilation was maintained with a permanent fan ventilator. The animals were fed water and purina laboratory diet ad lib. The composition of the diet supplied by the Ralston Purina Company was as follows:

Protein	22%
Fat	5%
Fiber	4%
Ash	7%
Calcium	1-3%
Phosphorous	0.9%
Corotene	3 P.P.M.
Vitamin A	10 I.U. per gram
Thiamin	5.5 I.U. per gram
Riboflavin	1 P.P.M.
Vitamin D	12 U.S.P.
Panacid	9 P.P.M.

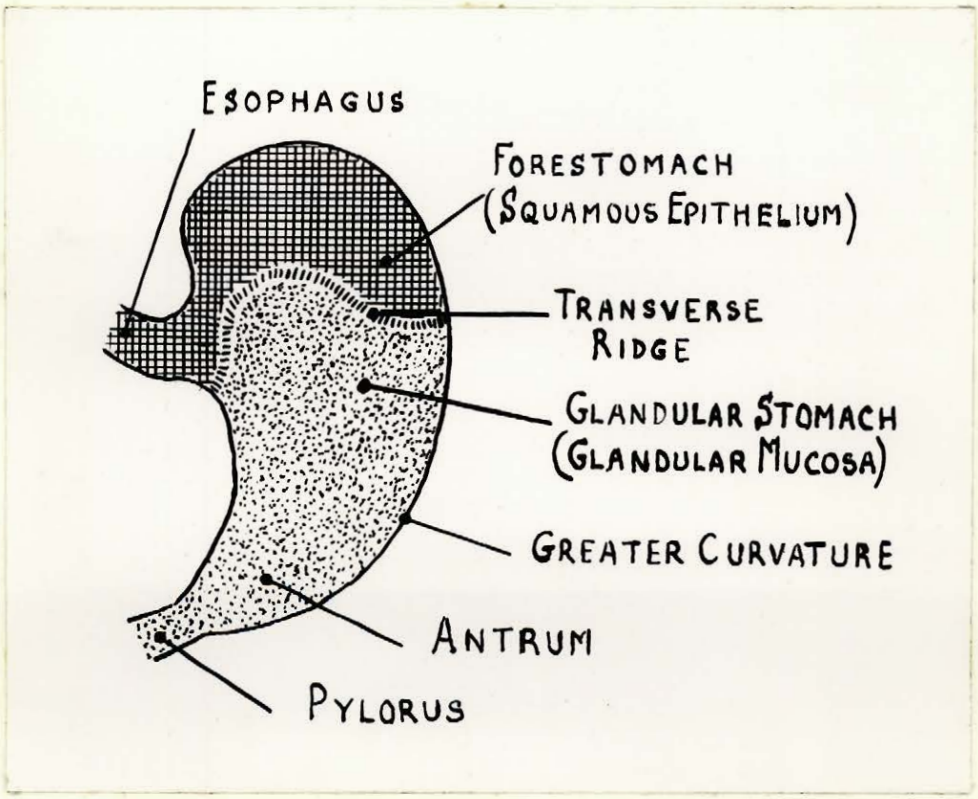
At the time of operation the animals were 2 to 3 months of age and weighed between 100 - 150 gms.

The anatomy of the rats stomach as with the other members of the genus Rattus, consists of 2 histologically different

sections. The forestomach is lined with squamous cell epithelium, while the remainder of the stomach is lined with glandular gastric mucosa. The 2 regions are separated by the limiting ridge.

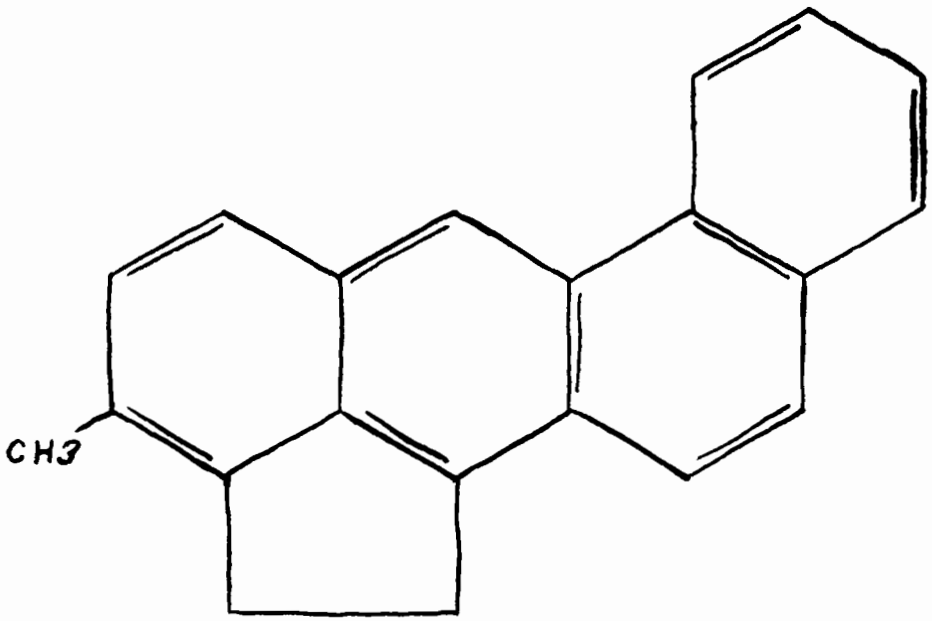
FIGURE IV.

Anatomy of Rat Stomach.



Carcinogen.

20-methylcholanthrene was the carcinogen utilized in this study. It is a polycyclic hydrocarbon.



20-methylcholanthrene

It is one of the most potent of the carcinogenic hydrocarbons (186) and its carcinogenic properties have been

extensively studied (131).

The vehicle for the carcinogen was a cotton thread, this method first being utilized by Andervont (172), in the production of pulmonary tumours. There are 3 reported series in which threads have been used previously in the stomach (152, 169, 170, 171).

To prepare the thread, the 20-methylcholanthrene (Brinkman and Co.) was placed in an open dish, and then heated over a bunsen burner until it was molton. A commercial type cotton thread, size number 40 was then drawn through the carcinogen, it was allowed to dry and then passed through again for a total of 3 passages. This procedure resulted in a thread, coated and impregnated with the carcinogen.

Operative Procedure:

The surgical procedure was carried out with clean but not sterile instruments. The rats were anaesthetized with intra-peritoneal nembutal (Abbot, veterinary, 60 mg. per cc.), dosage 5 mg. (0.08 cc) per 100 gms. body weight. The abdomen was then shaved and painted with merthiolate. The abdominal cavity was opened through a midline incision, the greater curvature of

stomach grasped and the stomach pulled into the wound.

Initially in the experiment the method of Stewart was used to insert the thread (169). In this method the carcinogenic thread is placed on a straight cutting needle, the needle is then inserted along the greater curvature of the stomach, running just distal from the limiting ridge to the pylorus. Although the stomach wall is rather thin it is possible to keep the thread in the submucosa and not to penetrate the mucosa or the serosa. The needle is then pulled through and the carcinogenic thread left, the 2 ends being trimmed.

This method was modified in the second half of the experiment, the thread was inserted in the same manner but 3 silk stay sutures were inserted. These sutures were inserted with a curved needle, including all the layers of the stomach wall and the carcinogenic thread in the ligature. The purpose of the stay suture was to hold the thread in place and hinder its passage into the stomach lumen.

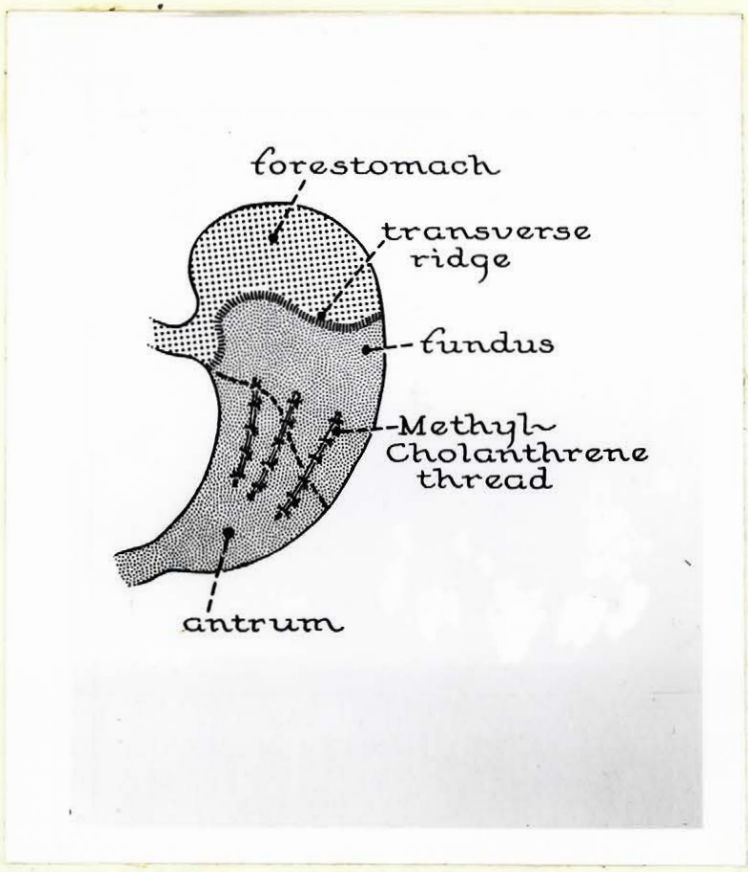


Figure V

Thread in Stomach Wall.

Following the insertion of the thread, the stomach was replaced in its bed. The abdominal wall being closed with a continuous silk suture, the rectus sheath and skin closed in separate layers.

It had been noted in previous experiments that the thread was usually gone within 60 to 100 days following its insertion,

presumably being sloughed into the stomach and being passed through the gastrointestinal tract. The technique of the stay suture was developed in an effort to hinder the sloughing of the thread. Also to ensure that the stomach would be exposed to the carcinogen for a longer period of time, the rats were reoperated upon at 3 and 6 months intervals following the original operation and new threads inserted. At the time of these subsequent procedures the previous threads were usually gone.

Transplantation:

Homotransplantation was attempted with a few of the tumours. The tumours which were removed at postmortem were ground up in a petri dish, moistened with saline and penicillin solution added. The tumour tissue was then drawn into a number 17 needle. The needle was fitted with a stilette which was used to draw up the material and also to expel it. The needle was then inserted through the skin into the subcutaneous tissue of the host, over the lateral pectoral region and the tumour tissue expelled by means of the stilette. Sterile technique was used.

Control Animals:

The rats in the control series were fed the standard purina diet and otherwise kept under the standard laboratory conditions. Cotton thread which had not been treated with methylcholanthrene was inserted into the stomach in a similar method as the carcinogenic threads. A second control series was setup in a similar manner but these animals were maintained on a vitamin B_{12} deficient diet.

Effect of Drugs and Diet:

In an effort to study the effect of various drugs and diets on the tumour induction some of the rats with threads inserted were subdivided into groups and treated in the following manner:

1. Cortisone. 0.2 cc of Cortone (Cortisone acetate U.S.P. Merck, Sharpe & Dohme) was given intramuscularly twice weekly. The injections were started 21 days after the first methylcholanthrene thread insertion. The second carcinogenic thread was inserted at 94 days and third thread at 227 days.

Stay sutures were not used in this group.

2. Supplemental Vitamin B_{12}: 1.0 cc (1000 mcgm) was given intramuscularly three times weekly (vitamin B_{12}, 1000 mcgm per cc, Paul Maney Laboratories X-619). This group was subdivided into 2 further groups, the first of which had the injection started 2 weeks after the thread insertions, the second and third threads inserted at 95 and 225 days but in which no stay sutures were used. The second group had the injections started at the time of the initial thread insertions, second and third threads at 90 and 180 days respectively and in this group stay sutures were utilized.

3. Vitamin B_{12} deficient: These animals were fed a vitamin B_{12} deficient diet. The diet was supplied by the Scientific Concentrate Company of Montreal and contained: Bone meal (sterilized), corn gluten meal, soybean oil cake meal, brewers yeast, yellow corn meal, common fine salt, trace mineral mixture, vitamin A concentrate, vitamin D-2, choline chloride.

As with the supplemental vitamin B_{12} group, this group was divided into 2. In the first group the diet was started 60 days

following the first thread insertion; second and third threads being inserted at 95 and 225 days and no stay suture being utilized. In the second group the diet was started at the time of the thread insertions, second and third threads being inserted at 90 and 180 days and stay sutures were utilized.

Serum B_{12} levels were obtained in the second groups of the supplemental and deficient B_{12} groups, the method of Cooper (187) being utilized. Blood was obtained from the ventricles at the time of autopsy, which was 14 months after the diet was started.

4. Lithospermum Diet: 200 gms of the ground up root of the plant Lithospermum ruderale was added to each 800 gms of purina (20% of diet). This diet was started 230 days prior to the first thread insertion. The second and third threads were inserted at 125 and 255 days respectively. Stay sutures were not used in this group.

5. Vitamin B Complex: 0.3 cc of B plex (Wyeth) was given intramuscularly 3 times weekly. Each cc of this preparation contained:

Thiamin	10 mg
Riboflavin	2 mg
Niacinamide	100 mg
Pyridoxine	5 mg
Calcium d-pantothemate	5 mg
Chlorbutol (preservative)	0.5%

The injections were started 1 week after the first thread insertion, second and third thread insertions at 93 and 213 days. No stay sutures were used.

6. Alcohol Washings: A stomach tube was passed and 1 cc of 100% ethyl alcohol was administered once a week. This procedure was begun 1 week after the initial thread insertion, second and third thread insertions at 96 and 236 days. Some of these animals had a second thread only at 163 days. No stay sutures were used.

7. Enterogastrone: 1.0 cc (20 mg) of enterogastrone hydrochloride (Armour Laboratories) was given intramuscularly daily (6 times weekly). The injections were started 1 month after the initial thread insertion, second and third threads at 90 and 120 days. No stay sutures were used.

8. Vitamin A deficiency: The animals were fed the following diet supplied by the Scientific Concentrates Company of Montreal.

Soybean oil meal, barley meal, rolled oats, wheat millings, brewer's dried yeast, bone meal, choline chloride, vitamin D-2, fine salt, trace mineral (marganese, iron, copper, zinc, cobalt).

This diet was started at the time of the original methylcholanthrene thread insertion, second thread inserted at 135 days, no third thread or stay sutures were used.

9. Intrinsic Factor: 1 gm of intrinsic factor was added to each kilogram of purine (Intrinsic factor concentrate without vitamin B_{12}, 42.85mg per U.S.P. oral unit when 15 gamma vitamin B_{12} added, 12,166 Organon Incorporated, Orange, New Jersey). This diet began 50 days after the first methylcholanthrene thread insertion, second thread inserted at 108 days, no third thread or stay sutures were used.

Postmortem Examinations:

The rats were examined once a month for the development of tumours, when tumours were present it was usually possible to palpate them through the abdominal wall. When the tumours were very large or when the animals appeared sick they were

sacrificed, a lethal dose of intraperitoneal nembutal being utilized. The stomach was then removed "en bloc", it was opened and examined for the presence of a thread, also tumours were looked for and the mucosa examined. The specimens were then placed in Sousa's solution. Histologic sections were prepared using Haematoxylin, Phloxine and Saffronin stains routinely and trichome, Van Gieson, reticulum galligo and Periodic Acid Schiff stains, when indicated. The remainder of the abdominal contents and thorax was examined grossly and where indicated specimens obtained for microscipic study. The experiment was terminated after 18 months, all surviving animals being killed at this time.

V.

RESULTS.

1. General Comments.

There were 440 animals operated upon which survived the immediate postoperative period, 116 (26.4%) malignant gastric tumours developed in these animals. The first tumour developed at 150 days. 410 of the 440 animals survived 150 days or longer, increasing the tumour incidence to 28.3%.

Only 1 spontaneous tumour developed, this occurred in the lung and was a reticulum cell sarcoma. Also in 2 cases, spindle cell sarcomas developed in the abdominal wall of animals which had no gastric tumours.

At autopsy there were frequent peritoneal adhesions found, the liver in particular frequently adhering to the stomach. The tumours, when present were usually in the anterior stomach wall and on the greater curvature. Grossly the tumours varied from minute nodules to up to 40 mm in diameter. The sarcomas tending to grow the largest. The tumours were firm, often calcified, with occasionally necrotic centres and obstruction of the pylorus occurred in a few cases.

The carcinomas tended to be white to grey in colour whereas the sarcomas appeared to be more vascular and had areas of haemorrhage. Several of the large sarcomas involved all the surrounding organs including the spleen, liver, pancreas, bowel and abdominal wall.

Grossly the carcinomas were occasionally ulcerated and in one case the whole glandular stomach was involved in a linitis plastica type of lesion.

Pulmonary abscesses and bronchiectases occurred in approximately 50% of the animals and were the chief cause of natural death. These infections appeared to develop slowly and did not occur in epidemics. The liver also on occasion contained white nodules which were abscesses. No gross metastases were seen, apart from several peritoneal implants in animals with sarcomas.

Figure VI

Adenoacanthoma Ulcerating Stomach

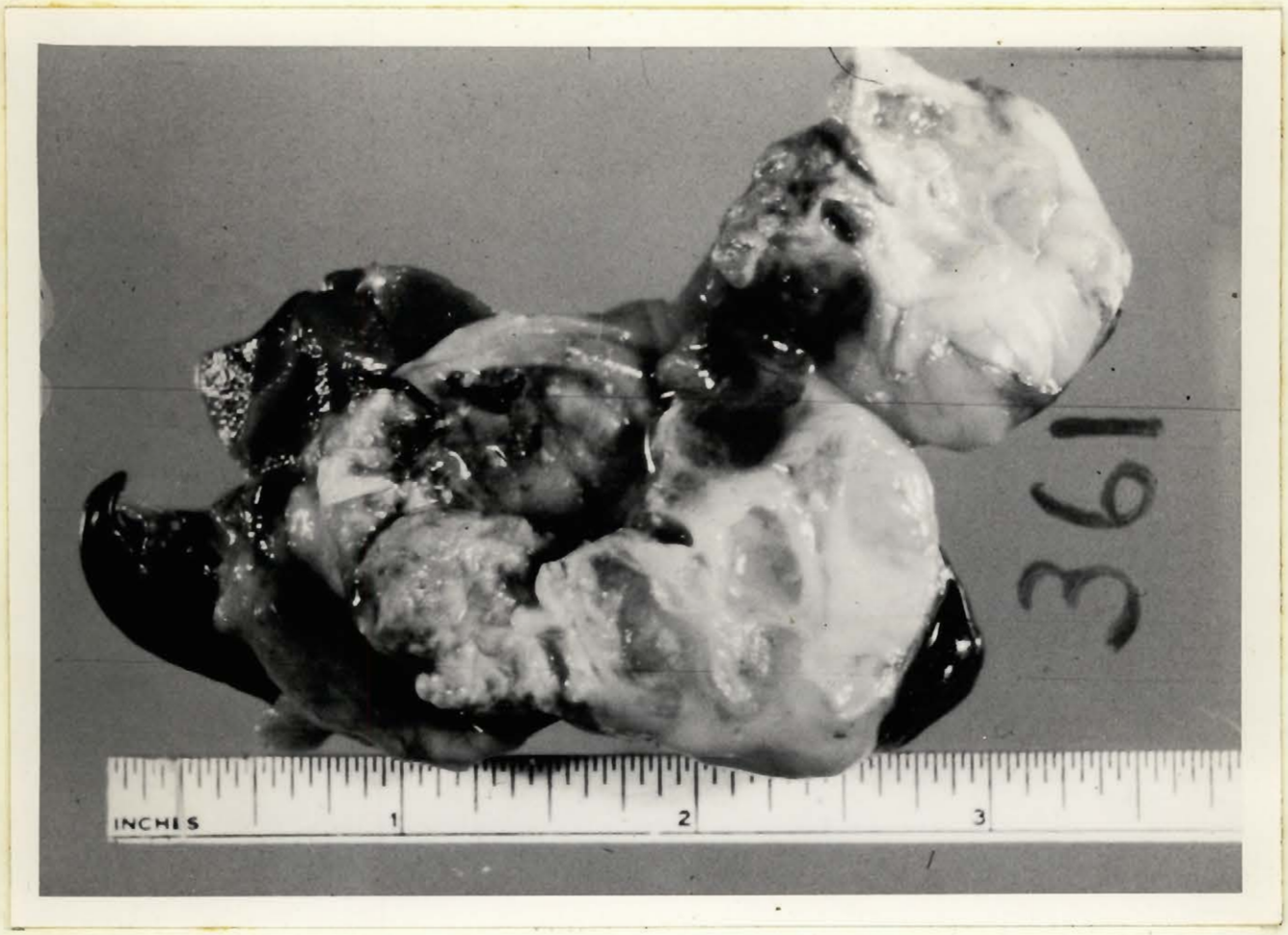


Figure VII

Gross Specimen: Spindle Cell Sarcoma

2. Results in Control Group.

The control group included those animals, in which no carcinogen was contained on the thread.

There were 36 control animals which came to autopsy, 11 of these were fed the normal purina diet and 25 were fed the Vitamin B_{12} deficient diet. No malignant tumours developed in these animals. In those animals fed the normal diet, there was chronic fibrosis present in 2 specimens, but no other abnormalities occurred. All of these 11 animals survived 320 days.

22 of the animals on the Vitamin B_{12} deficient diet survived more than the minimum tumour induction period of 150 days. The mean survival time of the group was 425 days. Diverticula were frequently encountered occurring in 9 (36%) cases.

These adenomatous diverticula were identical in appearance to those resulting from the threads containing carcinogen. Although the small early ones were found usually in the submucosa, the larger ones penetrated the muscle and extended into the subserosa as did the other diverticula. They frequently presented as nodules, up to 5 mm in diameter. Squamous metaplasia also occurred in the diverticula of the control group. Those associated with inflammation exhibited the anaplasia which was also seen in the

other series. However no elements of anaplasia were seen which could be considered to be truly malignant. The diverticula tended to occur in those animals which had lived the longest, the mean survival period of animals with diverticula was 453 days.

Apart from the diverticula no other differences were found between the 2 control groups.

3. Types of Tumours Produced.

The only benign tumour which occurred was the adenomatous diverticulum. It was possible to group the malignant tumours into 5 groups. The epithelial tumours occurring as adenocarcinomas and adenoacanthomas, which represented adenocarcinoma which had undergone squamous metaplasia. The sarcomas or connective tissue tumours were classified as spindle cell sarcomas, osteogenic or osteo sarcomas and undifferentiated or anaplastic sarcomas.

The incidence of each type in the total series was as follows:

Table I. Tumour Types.

Type of Tumour.	Number	%
Diverticulum	269 (in 374 specimens)	72.0%
Adenocarcinoma	35	8.5%
Adenoacanthoma	22	5.4%
Spindle Cell Sarcoma	32	7.8%
Osteogenic Sarcoma	12	2.9%
Anaplastic Sarcoma	15	3.6%
Total Malignant Tumours	116	28.3%
Total Specimens (surviving more than 150 days)	410	

4. Description of Tumours. - Diverticula.

Adenomatous diverticula were seen in 72% of the specimens in which carcinogenic threads were inserted. These lesions presented frequently as gross tumours and were often mistaken originally for malignancies. They appear as a proliferation of the glandular mucosa which develops into a mass of variable size, which may extend from the submucosa to the serosa. These lesions tend to penetrate the stomach wall, only 7% appeared to be confined to the submucosa, while 21% were seen penetrating into but not through the muscularis and 72% were in the subserosa. The cells and glands of the diverticula resembled the gastric mucosa although the crypts were not as deep. The glands tended to be

multilayered. Typically the cells were small, cuboidal and basophilic, mucous cells were frequently interspersed with the cuboidal cells. Parietal cells were seen in 6% and chief cells in 3%, the chief cells only being present where parietal cells were also present. This is in contrast to previous reports (152).

In those associated with inflammation, metaplasia and some anaplasia were seen, however there was no loss of polarity of the glands, chromatin and nucleolae were normal and abnormal layering was not present. They were all surrounded by a connective tissue capsule.

In 6% of those in the early series, the epithelial lining of the diverticulum was continuous with that of the stomach. The other 40% were separated from the gastric epithelium by connective tissue, although if more serial sections were done this percentage would be higher. But in some cases there appears to be a separation of the glandular tissue by inflammation or a reparative process.

The mouths of the diverticula were usually small with a narrow neck extending through the muscularis which then dilated to a large size in the subserosa. Squamous metaplasia occurred in 14%.

Frequently this was extensive with the formation of large cysts of keratin, in which case the diverticulum was then lined with squamous epithelium, sebaceous metaplasia was present in 1 specimen.

The diverticula did not occur with tumours. In the second vitamin B_{12} series, there were diverticula in 74% of the specimens without tumours, whereas 73% of adenocarcinomas and 86% of adenocanthomas had coexistent diverticula. When present with the tumour, more than half were in continuity with the tumour.

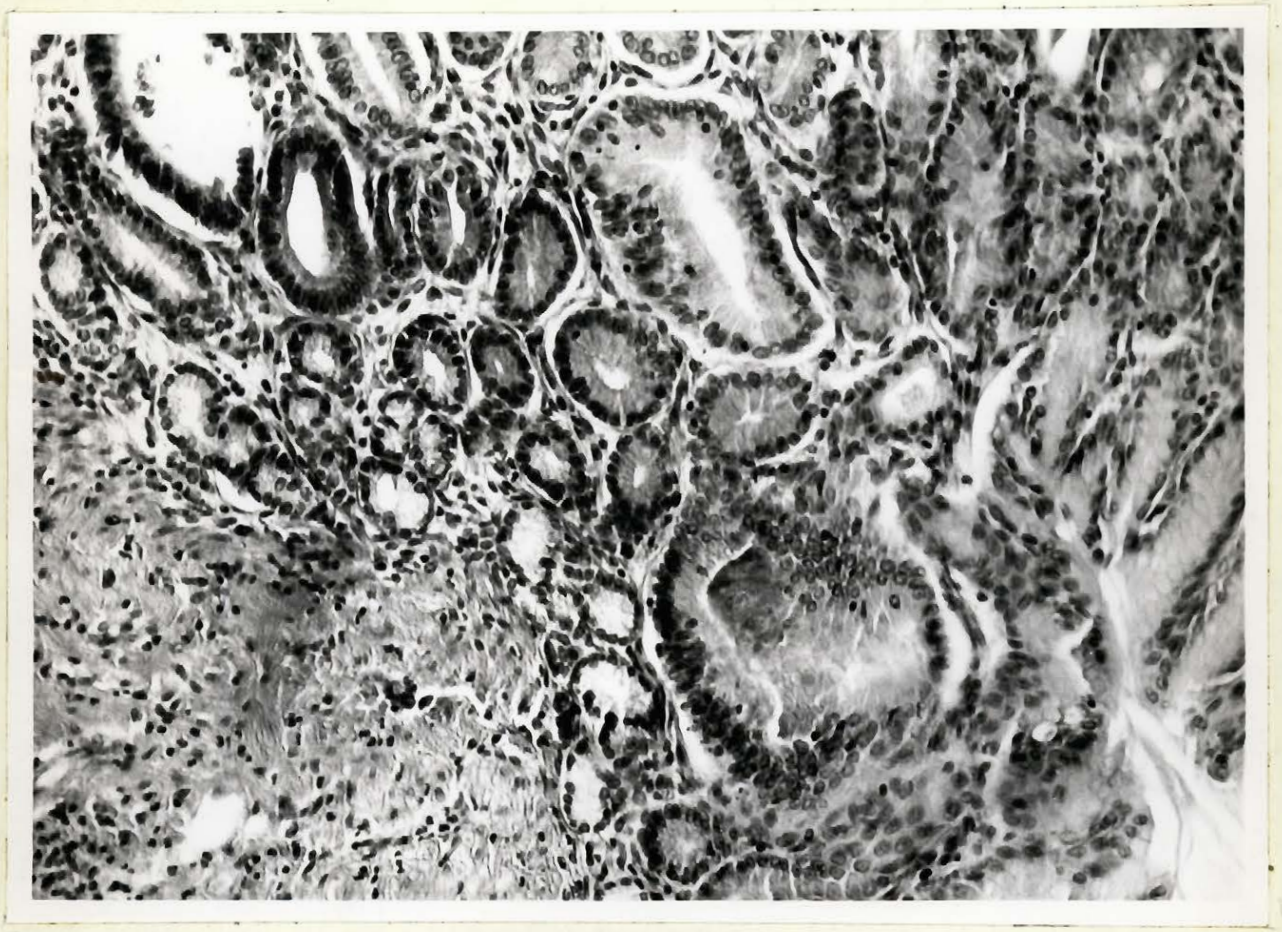


Figure VIII

Diverticulum x 75

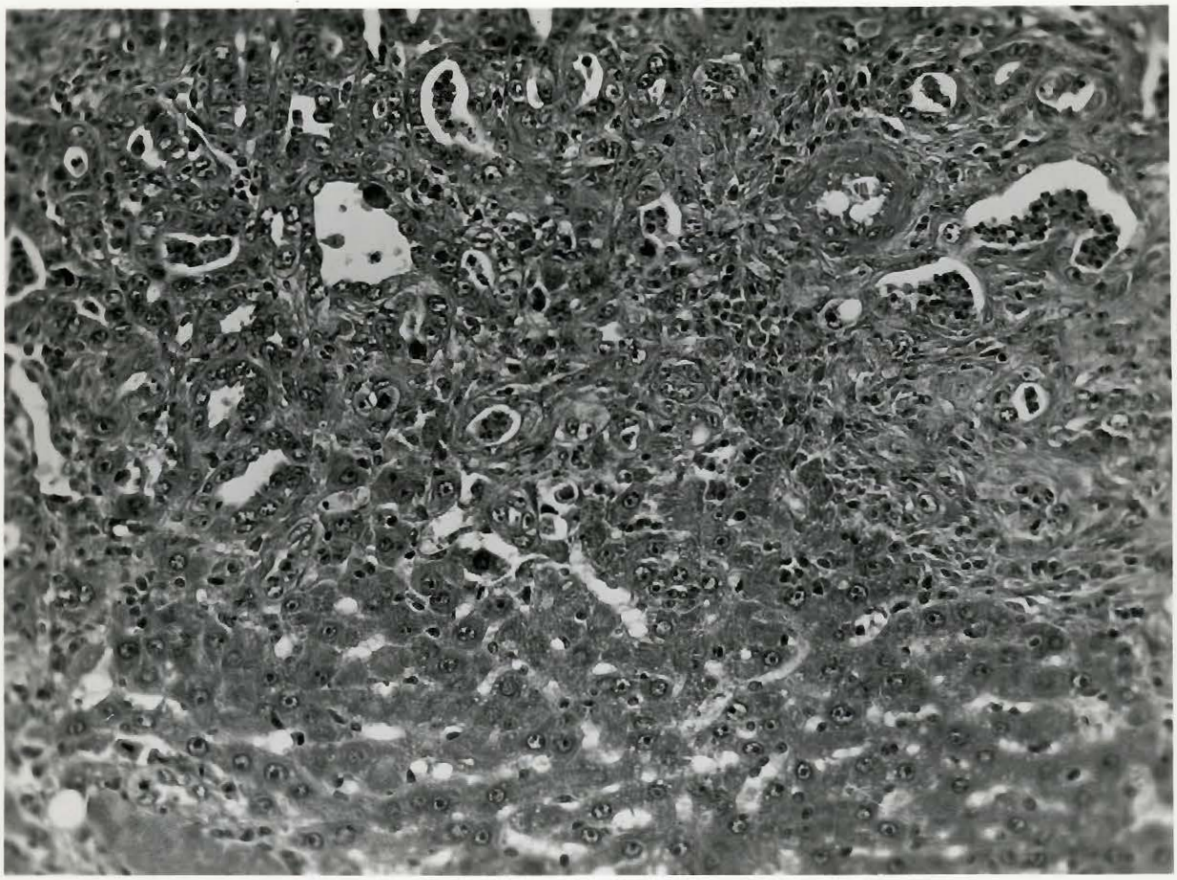


Figure IX

Adenocarcinoma Invading Liver x200

Adenocarcinomas.

The adenocarcinomas were generally well differentiated, with well developed acini. The glands were lined with cuboidal cells and were moderate in size with only occasional large glands. The glands tended to be irregular in shape, and the arrangement was quite variable, no tendency to wide separation or to close packing being exhibited. The glands frequently had no lumen, with 30% exhibiting carcinoma simplex. The stroma was slight in amount and tended to be coarse. The amount varied considerably in different areas of the same tumour. Hyalinized, sclerotic or calcified stroma was occasionally seen.

The epithelial cells of the glands did not tend to be highly anaplastic, half of the tumours exhibited only plus 1 anaplasia, and mitotic figures were not numerous. 3 of the tumours were highly anaplastic with numerous mitotic figures. The glands tended to be lined by a single layer of cells only, although some had up to 5 layers. The nuclear chromatin was usually even or regular and the nucleoli were as a rule not particularly large.

All of these tumours exhibited invasive qualities. The fat and connective tissue being frequently involved, muscle was seen to be involved in 70%, mucosa in 20%, liver in 41%, pancreas in

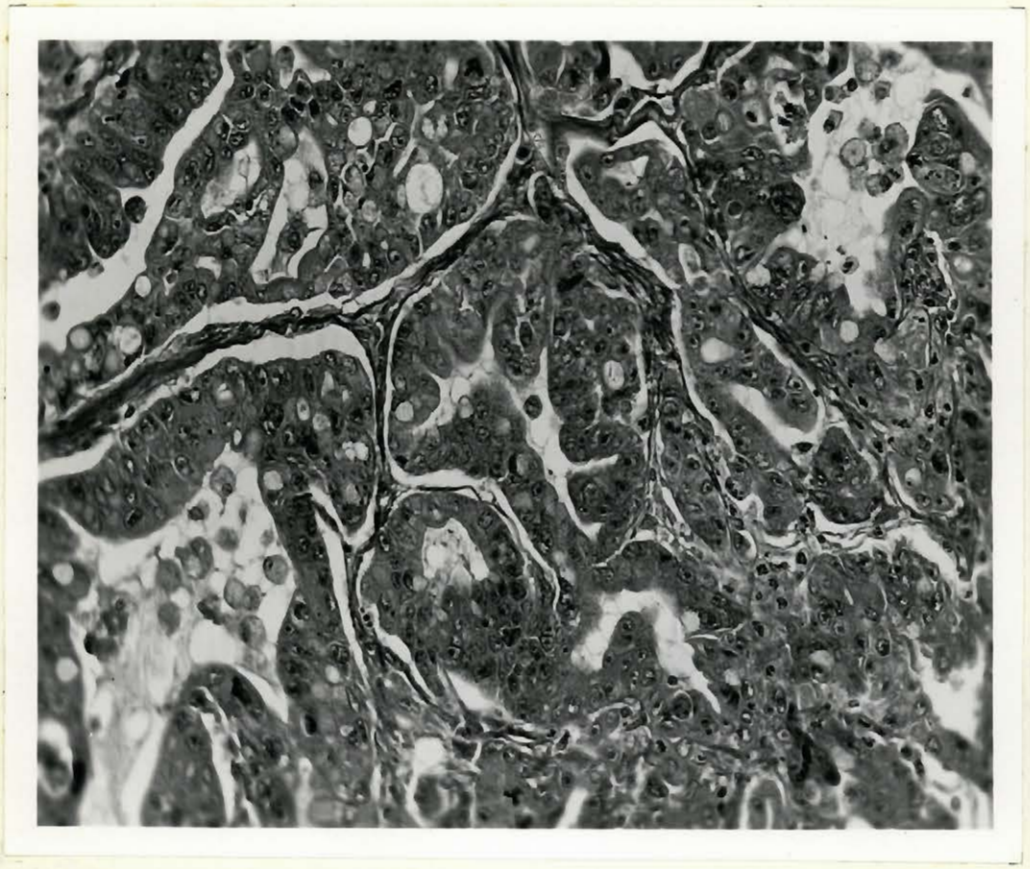


Figure X

Well Differentiated Adenocarcinoma x 200

12%, spleen in 1 case and an adjacent spindle cell sarcoma in 1 case. Invasion of the lymphatics and blood vessels was only seen in 1 tumour. Infiltration or invasion did not extend for great distances beyond the tumour mass. Although none had true capsules, the periphery in some cases was limited by a layer of collagenous tissue.

Inflammation was frequently present, occurring usually in the stroma rather than in the glandular portion. Necrosis was uncommon and ulceration was only seen in 2 cases.

The tumours were typically located in the subserosa but extended also into the submucosa and muscle in over half of the cases. Microscopically the size varied from 1 by 1.5 mm to greater than 15 mm in diameter. The average size was about 5 mm in diameter.

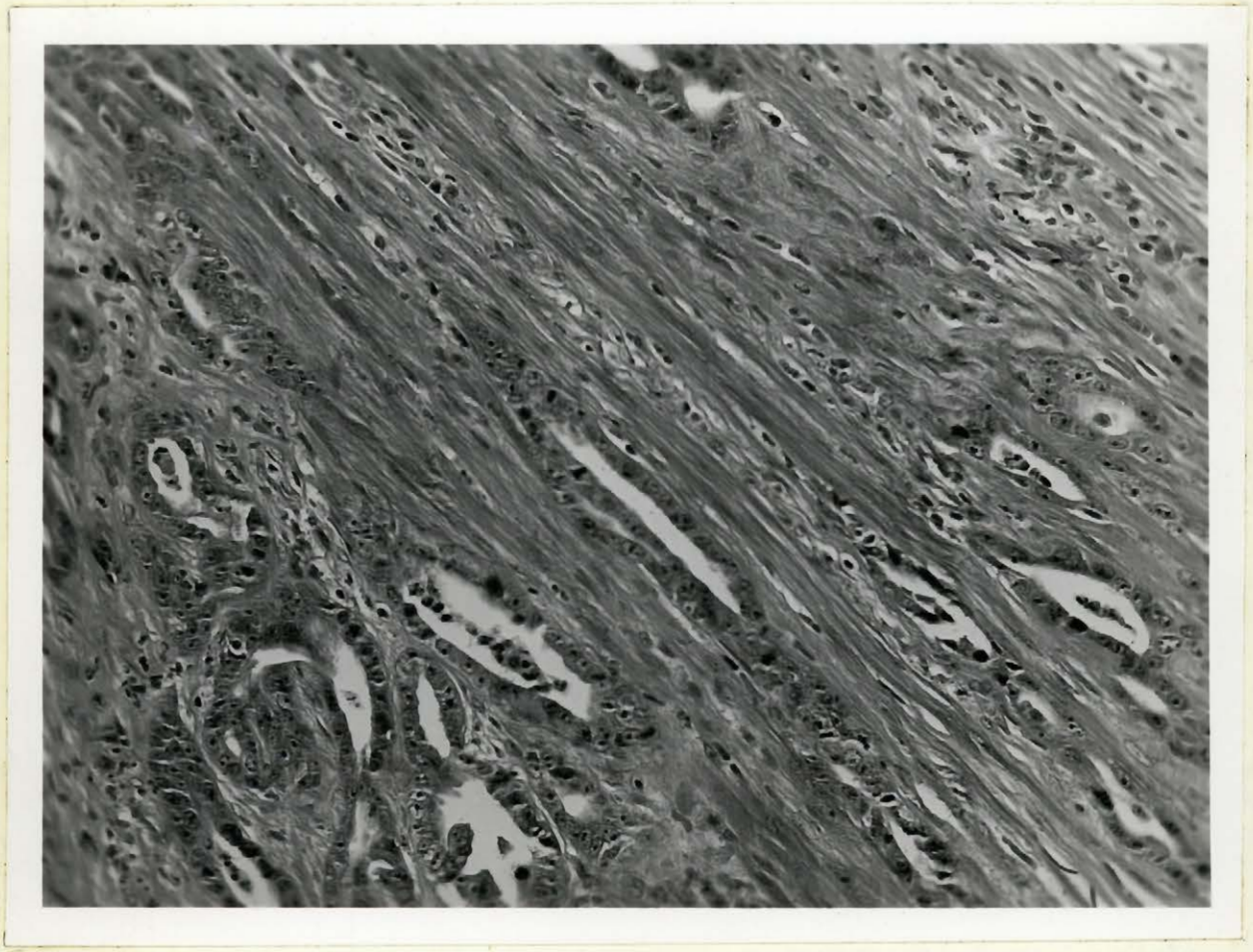


Figure XI

Adenocarcinoma Invading Muscle x 200

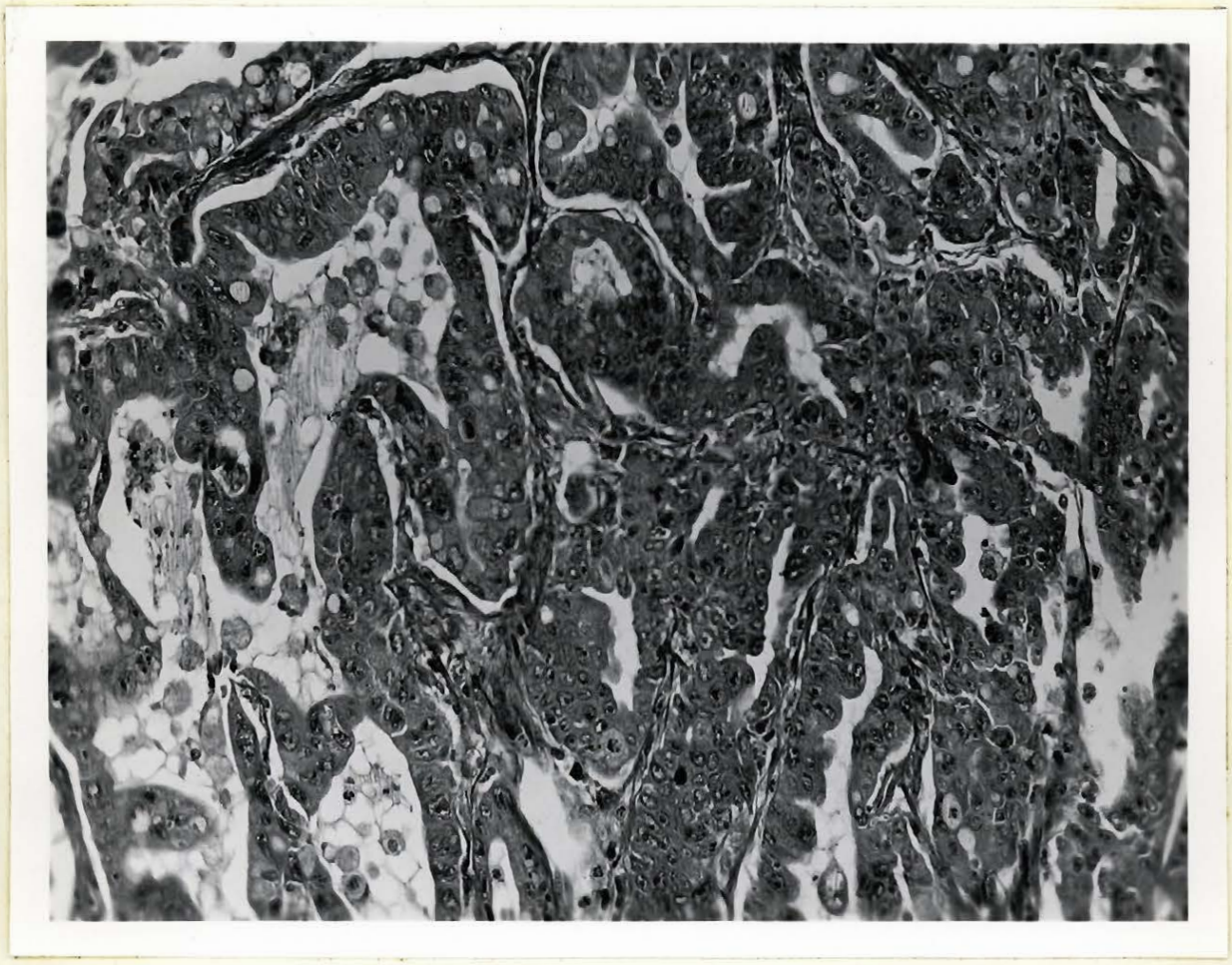


Figure XII

Adenocarcinoma x 200

Adenoacanthomas.

These tumours resembled the adenocarcinomas but there was a second element consisting of squamous epithelium. This squamous portion constituted from 5 to 99% of the tumour, with an average involvement of 50%. In some lesions the glandular elements could only be found with great difficulty, although in all cases there were some glands present, no pure squamous cell carcinomas occurred.

These tumours tended to be somewhat larger than the pure adenocarcinomas, the average diameter was approximately 9 mm. They were located in the same areas as the adenocarcinomas. Although they all showed invasive qualities, they did not tend to invade as readily as did the adenocarcinomas. The glandular and squamous portions exhibited similar invasive qualities. The pancreas was invaded in 6 cases, spleen in 2, liver in 9 and the skeletal muscle in 1 case.

The glandular elements were similar in structure to that seen in the adenocarcinomas. The most striking difference was the greater tendency to carcinoma simplex, 19 of 22 specimens demonstrating this. The glandular elements also showed more anaplasia with more mitosis and larger nucleoli than did that in

the pure adenocarcinomas.

The squamous portions occurred as squamous epithelium lining the glandular spaces. The clumps on islands of squamous cells were usually small and close together. The degree of keratinization was variable with a typical tumour only having small amounts. The squamous cells were moderately anaplastic although mitosis were not common. Chromatin varied from a regular to a moderately irregular pattern, nucleoli were also variable in size although excessively large ones were not typical. The stroma was scanty and coarse.

Inflammation was more common than in the adenocarcinomas, stromal inflammation occurring in 13 specimens, necrosis in 8 specimens and ulceration in 3.

In none of the specimens was the tumour adjacent to or continuous with the squamous epithelium of the forestomach.

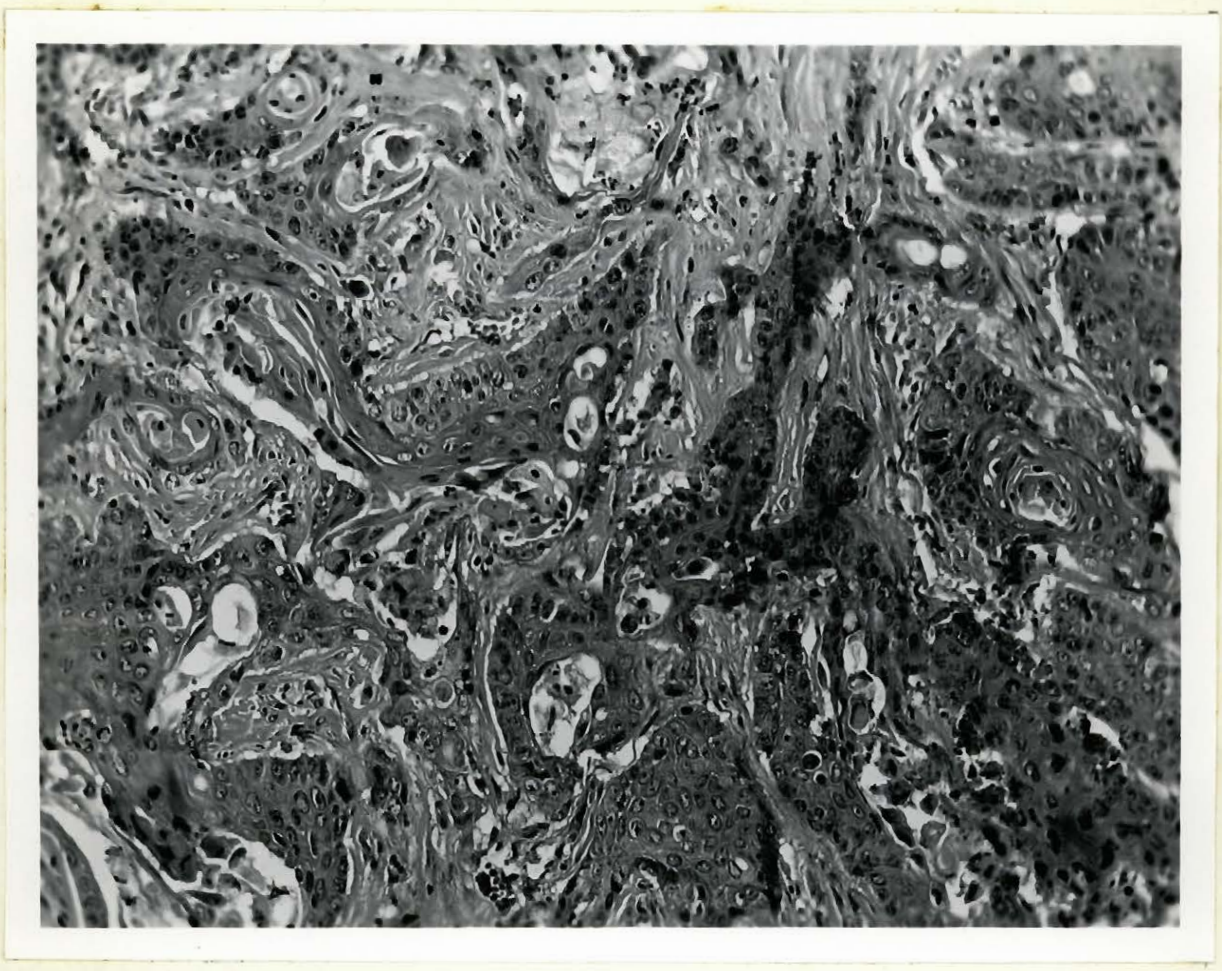


Figure XIII

Adenoacanthoma x 200

Sarcomas.

The sarcomas were classified into 3 types, spindle cell sarcomas, osteogenic and anaplastic types. Frequently these types were not present as pure types but blended from one type to another in the same tumour. The spindle cell type in particular occasionally contained elements suggesting a fibrosarcoma or leiomyosarcoma. However no neural elements suggesting a malignant schwannoma were seen.

A typical spindle cell sarcoma contained whorls of spindle-shaped cells between which contained variable amounts of collagen. The more anaplastic ones had less collagen, cells were larger, often irregularly shaped and had considerable amounts of acidophilic cytoplasm. The cells were variable in size although not typically large. This tumour type was not as anaplastic nor did it exhibit as many nuclear abnormalities as did the other sarcomas.

The spindle shaped cells ran in parallel lines separated by relatively dense stromas. A methylcholanthrene thread induced subcutaneous tumour in the same strain of rat was similar in structure, although the stroma was not as dense.

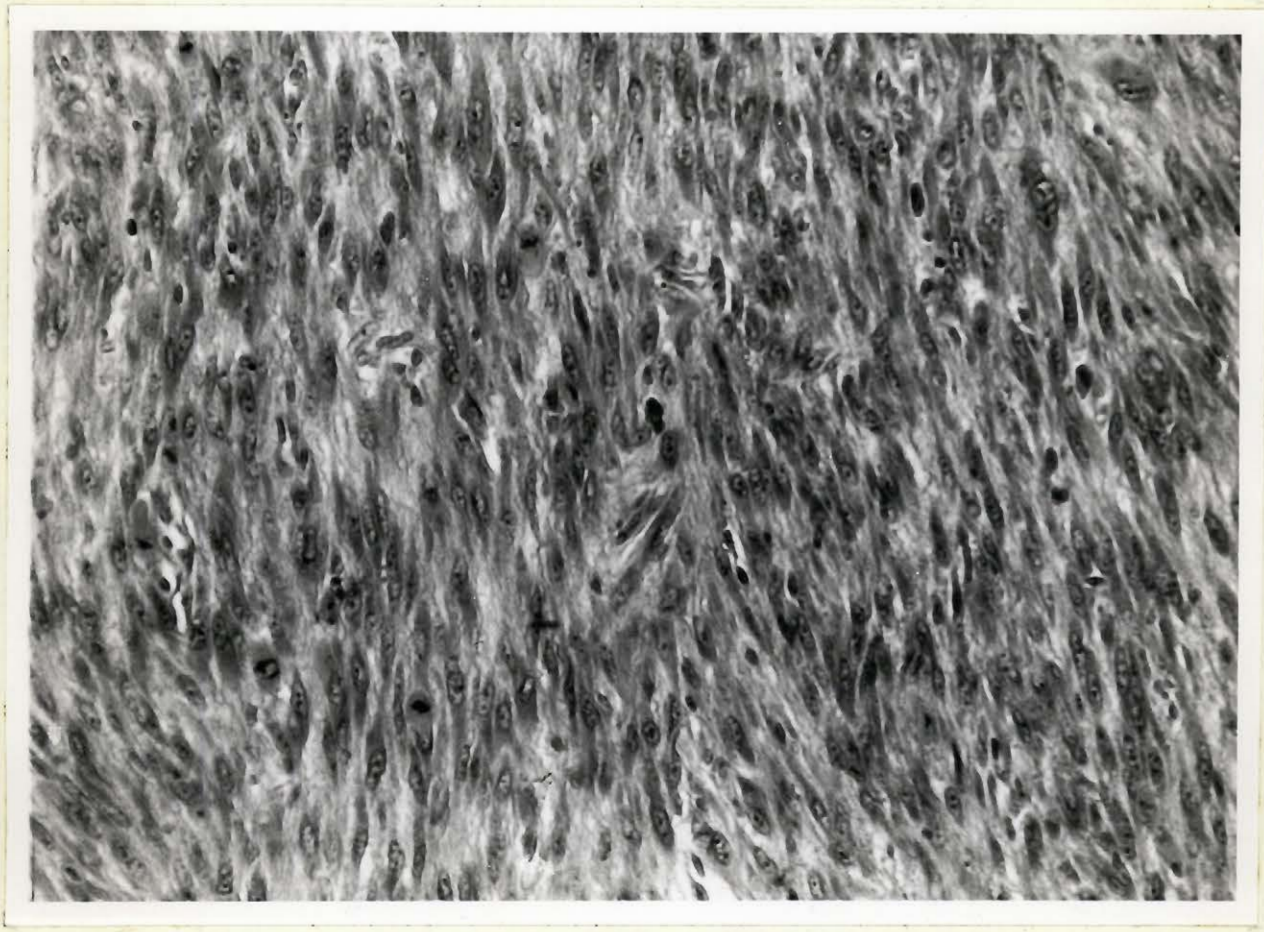


Figure XIV

Spindle Cell Sarcoma x 200

Those tumours classified as osteogenic sarcomas appeared more anaplastic than did the spindle cell tumours. They were characterized by areas of bone in or adjacent to which there were malignant appearing osteoblasts. These cells were round, hyperchromatic, showing a moderate loss of polarity and frequent mitosis. The bone, or frequently just osteoid, was in bands or bars which were separated by these anaplastic osteoblasts. These were frequently mixed tumours, with elements of the spindle cells and or the anaplastic sarcomas.

The anaplastic tumours consisted of solid masses of large, irregular bizarre cells, often multinucleated and with well defined acidophilic cytoplasm. Mitotic figures were frequent, large nucleoli were often seen and chromatin was irregular. Stroma was slight, patterns suggestive of anaplastic carcinoma was demonstrated when groups of these large cells were found contiguous one to another.

Occasionally the sarcomas were associated with diverticula, although not as frequently as were the carcinomas. This was due in part to the fact that sarcomas did not arise from the diverticula and if the 2 lesions were present in separated areas in the same

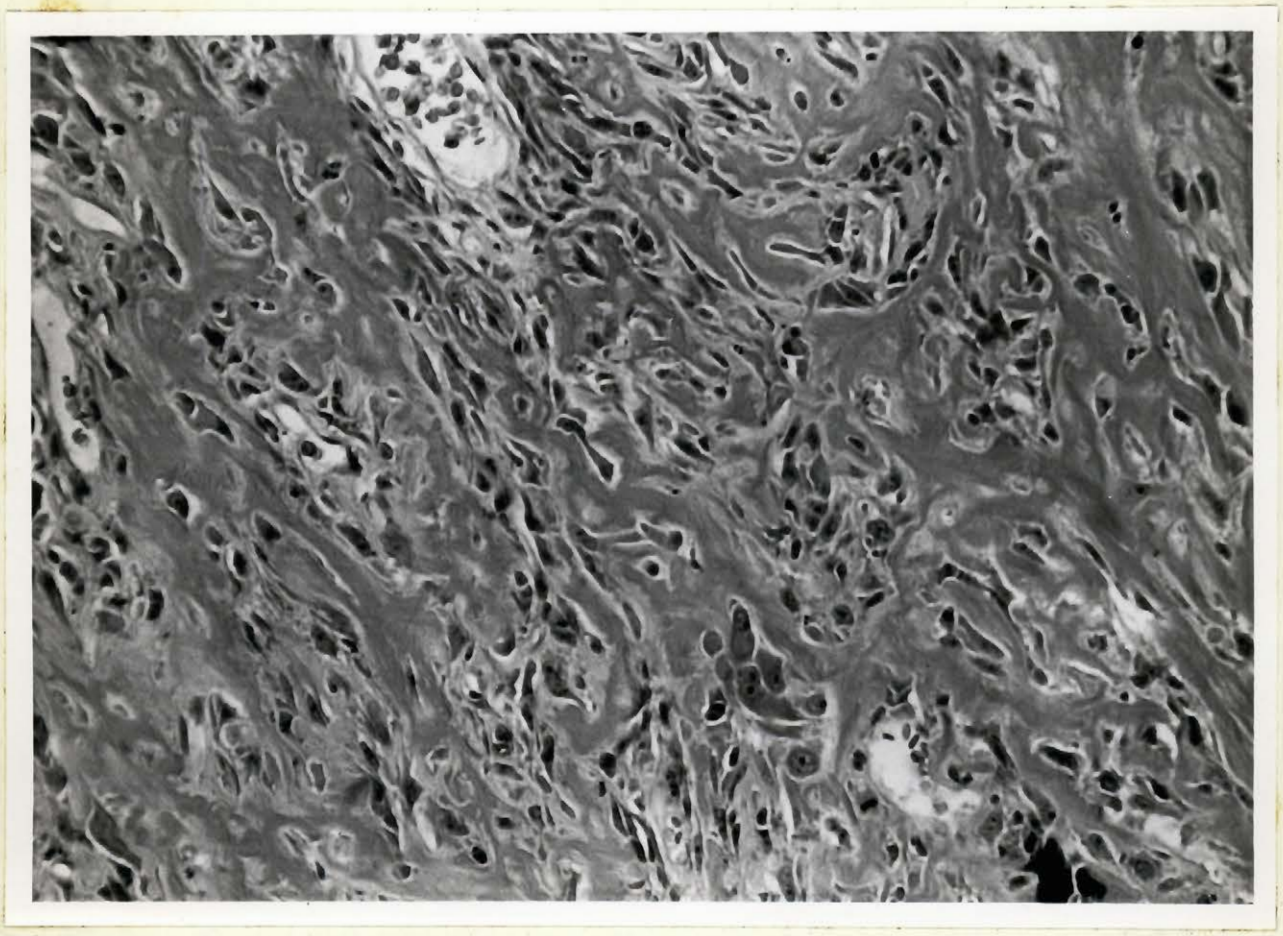


Figure XV

Osteogenic Sarcoma x 200

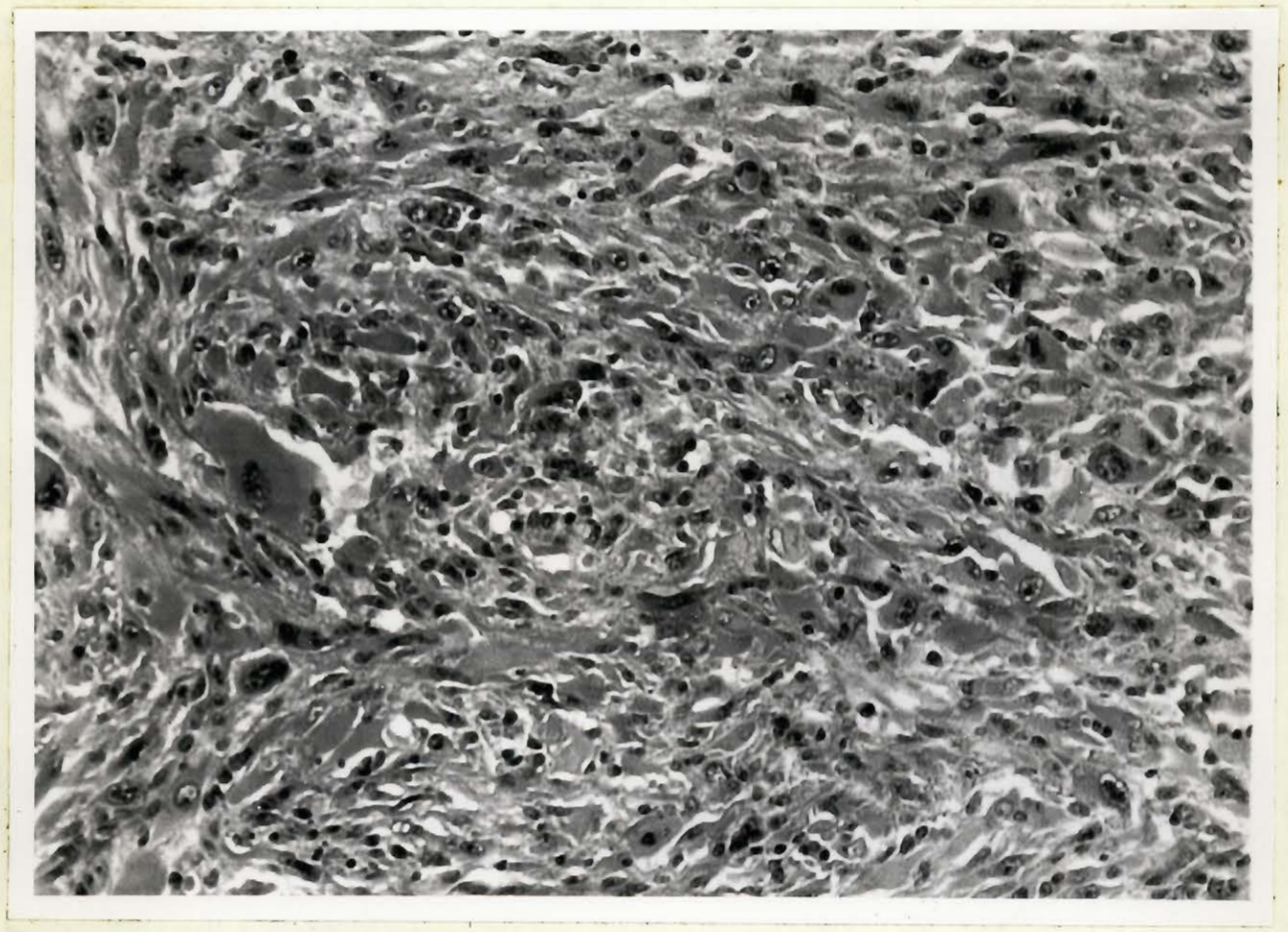


Figure XVI

Anaplastic Sarcoma x 200

stomach, only the sarcoma would be sectioned. The sarcomas were larger than the carcinomas, up to 30-40 mm in diameter and also invaded more extensively than the carcinomas.

5. Results in Groups with Modifying Factors.

A. Carcinogenic Thread without modifying Factors.

In the first group which were maintained on a normal diet and in which no drug was injected, 2 malignant tumours developed in 29 animals; 1 adenoacanthoma and 1 adenocarcinoma. The mean survival period was 320 days with a range of 10 days. There were 6 gross threads present at autopsy.

B. Effects of Alterations in Vitamin B_{12}.

The first group receiving supplemental vitamin B_{12}, in which no stay sutures were used, produced 1 adenocarcinoma, 22 diverticula and 1 pseudosarcoma in 30 animals. This latter tumour resembled the spindle cell sarcoma but there was no invasion, anaplasia was minimal and there appeared to be a marked degree of inflammation. The mean survival period was 298 days with a range of 110 days, threads present grossly in 7 cases.

The first group receiving a vitamin B_{12} deficient diet, in which no stay sutures were used, had 2 adenocarcinomas and 2 spindle cell sarcomas in 22 animals. Diverticula occurred in 14 animals and 9 threads were present at autopsy. The mean survival period was 175 days with a range of 30 days.

C. Effect of Lithospermum ruderale.

In the group receiving the lithospermum diet, of 18 animals only 12 survived more than 150 days. 1 adenocarcinoma developed, 10 diverticula, 10 ulcers of the gastric mucosa and 10 specimens showed bone formation. The mean survival period was 220 days with a range of 220 days, threads were present grossly in 2 cases. The animals did not gain weight as rapidly as did the other series.

D. Effect of Vitamin B complex.

In the group injected with vitamin B complex, 1 adenocarcinoma, 1 spindle cell sarcoma and 1 osteogenic sarcoma developed in 31 animals. There were 18 diverticula, 3 ulcerated stomachs and the mean survival period was 295 days with a range of 87 days, threads were present grossly in 6.

E. Effect of Alcohol Washings.

The animals receiving gastric washings with alcohol are in 2 groups although they have been charted as 1 group. The first group had 3 carcinogenic thread insertions while the second group had only 2 insertions. In the first group there were no malignant lesions in 17 animals, 15 surviving more than 150 days. Sebaceous metaplasia occurred in 1 case and diverticula in 9. The mean survival period was 269 days with a range of 200 days and threads were present in 7 cases. In the second group 1 adenoacanthoma and 1 fibrosarcoma developed in 28 animals, 22 of which survived more than 150 days. Diverticula occurred in 20 cases, the mean survival period was 182 days with a range of 160 days and threads were present in 3 cases. In none of these 2 groups was there evidence of gastritis or ulceration from the alcohol.

F. Effect of Enterogastrone.

In the animals injected with enterogastrone, 1 adenocarcinoma developed in 25 cases, 22 of which survived more than 150 days. There were 16 diverticula, the mean survival period was 260

days with a range of 200 days and threads were present grossly in 6 cases.

G. Effect of Vitamin A Deficiency.

The animals fed a vitamin A deficient diet produced 2 spindle cell sarcomas and 8 diverticula in 18 animals. The mean survival period was 155 days with a range of 15 days and threads were present grossly in 8 cases. There were no microscopic abnormalities suggestive of vitamin A deficiency.

H. Effect of Intrinsic Factor.

In the animals fed intrinsic factor, almost 50% of 13 animals surviving 150 days developed malignant tumours. There were 3 adenocarcinomas, 1 adenoacanthoma, 1 spindle cell sarcoma and 1 osteogenic sarcoma. 6 diverticula developed, the mean survival period was 373 days with a range of 403 days. No record was made of the number of threads present at autopsy.

I. Effect of Vitamin B_{12}, modified method of thread implantation.

In the second series of animals receiving vitamin B_{12} injections, in that series in which stay sutures were utilized, there were 36 malignant tumours in 81 specimens, all surviving

more than 150 days. The tumours were adenocarcinomas 6, Adenoacanthomas 10, spindle cell sarcomas 10, osteogenic sarcomas 2, anaplastic sarcomas 8 and combinations of these or multiple tumours 5. The mean survival period was 400 days with a range of 414 days. Serum vitamin B_{12} levels done on 3 of these animals were as follows:

5037.04 micro micrograms per ml.
1400.00 " " " "
1200.00 " " " "

The second series of animals receiving a vitamin B_{12} deficient diet produced 56 malignant tumours in 92 animals, all of which survived more than 150 days. The tumours were adenocarcinomas 18, adenoacanthomas 9, spindle cell sarcomas 14, osteogenic sarcomas 8, anaplastic sarcomas 7 and combinations or multiple tumours in 8 specimens. The mean survival period was 360 days with a range of 369 days. Serum vitamin B_{12} levels done on 4 of these animals were as follows:

232.26 micro micrograms per ml.
252.63 " " " "
355.50 " " " "
767.00 " " " "

The observed microscopical abnormalities in the various groups included diverticula, squamous metaplasia, inflammation, bone formation, ulceration, and the described types of malignant tumours. There were no other striking microscopical changes seen. Also apart from the variations mentioned, no striking differences were seen which characterized any one group. In none of the groups did the administered drug or diet give rise to any observed metabolic abnormalities. The animals on the Lithospermum diet did not gain weight as rapidly as did the other groups but no other nutritional disturbances were observed.

Table II Effect of Modifying Factors on Tumour Induction.

Tumour Type	Carcinogen only	Cortisone	Supplemental Vit. B12	Deficient Vit. B12	Lithospermum diet
No. of Specimens (living more than 150 days)	29	23	30	22	12
Diverticula	23	19	22	14	10
%	79.3	82.6	73.3	63.6	83.3
Adenocarcinoma	1	1	1	2	1
Adenoacanthoma	1	-	-	-	-
Spindle Cell Sarcoma	-	1	-	2	-
Anaplastic Sarcoma	-	-	-	-	-
Osteogenic Sarcoma	-	-	-	-	-
Multiple Tumours	-	-	-	-	-
Total Tumours	2	2	1	4	1
% Tumours	6.9	8.7	3.3	18.1	8.3

Table II Effect of Modifying Factors on Tumour Induction (continued).

Tumour Type	Supplemental Vit. B. complex	Alcohol washings	Enterogastrone	Deficient Vit. A	Intrinsic Factor
No. of Specimens (living more than 150 days)	31	37	22	18	13
Diverticula	18	29	16	8	6
%	58.7	78.4	72.7	44.4	46.1
Adenocarcinoma	1	-	1	-	3
Adenoacanthoma	-	1	-	-	1
Spindle Cell Sarcoma	1	1	-	2	1
Anaplastic Sarcoma	-	-	-	-	-
Osteogenic Sarcoma	1	-	-	-	1
Multiple Tumours	-	-	-	-	-
Total Tumours (excluding Multiple)	3	2	1	2	3
%	9.7	5.4	4.5	11.1	46.1

Table II Effect of Modifying Factors on Tumour Induction (continued).

Tumour Type	Supplemental Vit. B12 2nd series	Deficient Vit. B12 2nd series	Total Vit. B12 2nd series	Total early series	Total all series
No. of Specimens (living more than 150 days)	81	92	173	237	410
Diverticula	-	-	-	165	-
%	-	-	-	69.6	-
Adenocarcinoma	6 (7.4%)	18 (19.6%)	24 (13.9%)	11 (4.6%)	35 (8.5%)
Adenoacanthoma	10 (12.3%)	9 (9.8%)	19 (11.0%)	3 (1.3%)	22 (5.4%)
Spindle Cell Sarcoma	10 (12.3%)	14 (15.2%)	24 (13.9%)	8 (3.4%)	32 (7.8%)
Anaplastic Sarcoma	8 (9.9%)	7 (7.6%)	15 (8.7%)	-	15 (3.6%)
Osteogenic Sarcoma	2 (2.5%)	8 (8.7%)	10 (5.8%)	2 (0.8%)	12 (2.9%)
Multiple Tumours	5 (6.2%)	8 (8.7%)	13 (7.5%)	-	13 (3.2%)
Total Tumours (excluding multiple	36	56	92	24	116
%	(44.4%)	(60.9%)	53.2%)	(10.1%)	(28.3%)

6. Retention of Carcinogenic Threads.

The carcinogenic threads were found at autopsy in 110 of 415 stomachs, (26.5%). When found, these threads were loosely embedded in the mucosa and either fell into the lumen or could be pulled out with ease. No threads were analyzed at the time of autopsy for quantity of carcinogen. Occasionally 2 threads were found. In no case was the thread found free in the peritoneal cavity.

There was a slightly higher percentage of threads found in the later Vitamin B_{12} series in which stay sutures were used than in the earlier series (30.0% as compared to 24.0%).

In the later vitamin B_{12} series there was only a slight tendency to find the threads in stomachs which contained tumours (34%), rather than in the stomachs without tumours (30.7%). In this series the percentage of each type of tumour which had associated gross threads was as follows:

Tumours Associated with Gross Threads (B_{12} Series).

Adenocarcinoma	42%
Adenoacanthoma	37%
Spindle Cell Sarcoma	33%
Osteogenic Sarcoma	30%
Anaplastic Sarcoma	20%
Multiple Tumours	30%
All Tumours	34%
All Specimens	30%

Microscopically the threads could often be seen although it was impossible to differentiate the silk stay sutures from the carcinogenic thread, as the embedding process dissolved the carcinogen. In the first series 76% of the first 194 specimens examined contained threads, in the later series the threads were removed when found prior to sectioning, as they damaged the microtome. In the 34 analyzed adenocarcinomas of the later vitamin B_{12} series, the thread was seen microscopically in 10 specimens (29%), being adjacent to the tumour in 3 cases only. In the 22 analyzed adenoacanthomas the thread was only present in 3 cases, being adjacent to the tumour in 2 of these.

The threads were located in the subserosa in 75%, muscularis in 19%, submucosa in 5%, pancreas in 3% and in one a thread was found in the liver. The threads were usually associated with a giant cell reaction, but only occasionally was there evidence of encapsulation. The strands of the threads sometimes remained close together, with little or no infiltration of cells between the fibres, although often the strands were separated by chronically inflammed collagenous tissue.

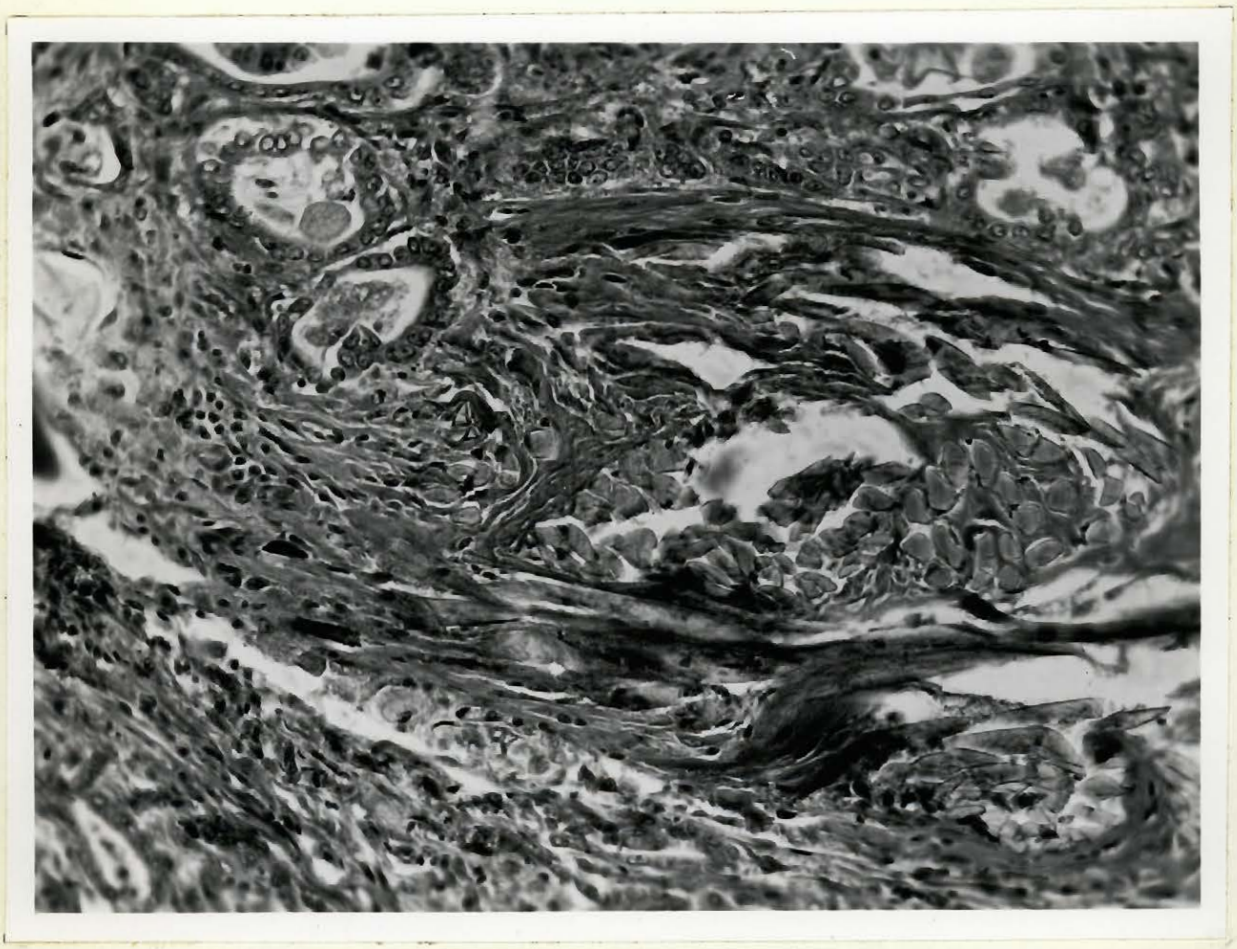


Figure XVII

Methylcholanthrene Thread in Submucosa x 200

7. Bone Formation.

In the early series there were only 6 specimens showing bone formation, 2 of which were seen as sarcomas. In the last 2 vitamin B_{12} series bone was seen in 16% of 173 specimens. In the supplemental vitamin B_{12} group, 15% of 81 specimens contained bone, 2 of the 12 of which were present as sarcomas. In the vitamin B_{12} deficient group, 17% of 92 specimens contained bone, 8 of the 16 of which were present as sarcomas. 9 of the 28 examples seen in the B_{12} series were present in stomachs which had no malignant lesions.

8. Multiple Tumours.

There were no multiple tumours seen in the groups prior to the second B_{12} series. However in the second vitamin B_{12} groups, 13 of the 116 tumours contained 2 separate tumours. The combinations were:

Tumour Types	#
Adenoacanthoma with spindle cell sarcoma	3
Osteogenic sarcoma with anaplastic "	3
Adenocarcinoma with osteogenic "	2
Spindle Cell sarcoma with anaplastic "	2
Adenocarcinoma with fibrosarcoma "	1
Adenoacanthoma with osteogenic "	1
Adenoacanthoma with anaplastic "	1

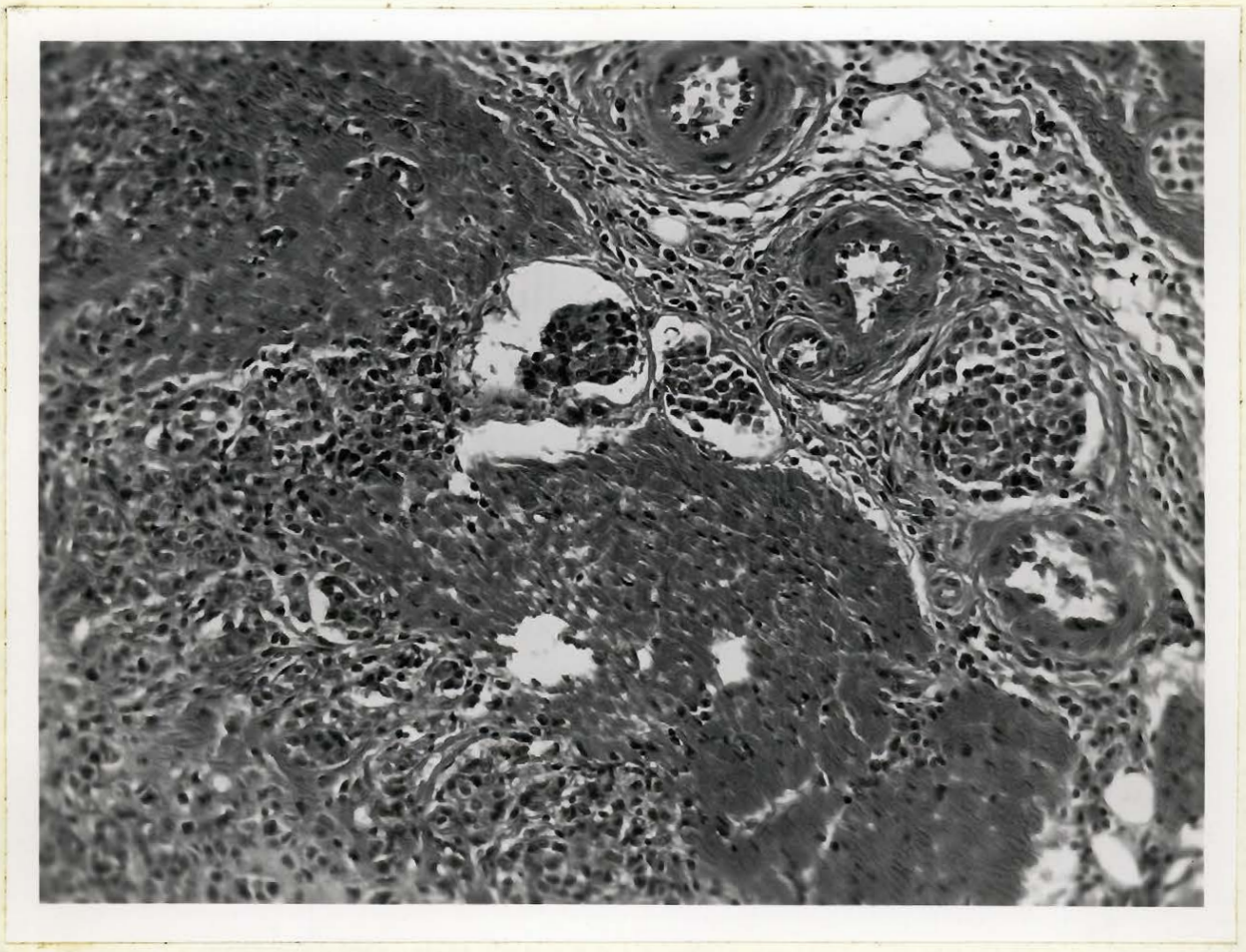


Figure XVIII

Adenocarcinoma Invading Lymphatics and Blood Vessels
x 200

As has been mentioned elsewhere, the sarcomas frequently contained elements of several types, but these multiple tumours all appeared to be separate primaries and not variable patterns of the same tumour.

9. Transplants.

Homotransplantation of the tumours was done with 35 tumours, (3 were multiple tumours). The classification of the primary tumours was:

	#
Adenocarcinoma	7
Adenoacanthoma	7
Spindle Cell Sarcoma	12
Diverticula	2
Osteogenic sarcoma	3
Anaplastic sarcoma	4

Only 3 of the transplants survived as tumours; 2 anaplastic tumours each grew with a similar structure as the primary and 1 combined osteogenic and spindle cell sarcoma grew as a spindle cell sarcoma. The remainder were destroyed in inflammatory processes and abscess formation.

10. Survival Times.

The mean survival periods following the first thread insertion have been tabulated in Table (III).

The early series up to and including the intrinsic factor series are included in 1 group. The mean survival times of the normals (those without malignancies) have not been calculated for this group.

Table III Survival Times following Thread Insertions (mean value).

Tumour Type	First Series (prior to B12)	Supplemental Vit. B12	Deficient Vit. B12	Total Vit. B12	All Groups Total	All Groups Range (Days)
Adenocarcinoma	297.4	375.3	392.6	388.3	359.8	393
Adenoacanthoma	317.0	412.4	362.3	388.7	378.9	346
Spindle Cell Sarcoma	213.1	403.7	338.5	365.6	327.7	352
Osteogenic Sarcoma	-	-	405.0	422.1	411.0	197
Anaplastic Sarcoma	-	424.4	369.8	398.0	398.0	133
Multiple Tumours	-	410.4	391.2	398.0	398.0	
Normals	-	390.9	329.2	363.4	-	
Total Specimens	-	399.7	329.0	376.4	-	

VI

DISCUSSION

A. General Factors Affecting Tumour Induction.

The induction of 43 carcinomas in 173 animals (24.8%) is a much higher incidence than has been reported by any previous method. Previously the highest reported series was 7 carcinomas in 273 rats (167), which occurred with the injection technique. This high rate only occurred in the last vitamin B_{12} series after the method of thread insertion had been modified.

The relative success of this method can be attributed to several factors, the stay sutures probably being the most important. The tumour induction rate increased greatly with the intrinsic factor series which was the first series in which the stay sutures were used. The factor of second importance is that the animals were kept for a relatively long period of time. The second B_{12} series were followed for 18 months which is half the life span of a rat, whereas the earlier series were not kept for as long a period. The reinsertion of the threads at 3 and 6 months was probably of less importance, for this technique was used in all the animals although the tumour induction rate in the first series was very low. Since the tumour induction rate

was low in the first series, this high rate of tumour induction in the last group was due to the method of inserting the carcinogen and the time of followup and not because the rats belonged to a cancer susceptible strain.

The factor of prime importance in gastric tumour induction is that the carcinogen remainsin situ for as long a period as possible. Although Howes & de Oliveira (152) showed that carcinogenesis can result after only 60 days of exposure to the carcinogen, undoubtedly malignant degeneration will occur in a higher percentage if the carcinogen remains in situ for a longer period.

The reinsertion of the thread on 2 occasions does not appear to be of great importance. Perhaps this is because at reinsertion the new thread is not placed in exactly the bed of the old one. Therefore instead of continuing the process begun by the first thread it must start anew with normal tissue.

These gastric tumours develop after a relatively long period of exposure to the carcinogen. The mean induction period for the adenocarcinomas was 360 days which is about one third the life span of a rat. The high rate of tumour induction in the last series was due in part to the relatively long period for which the animals were

kept. The first groups were not kept for 18 months as were the first B_{12} series and neither were the reported series in the literature. The time factor must be considered an important factor in the production of gastric adenocarcinomas. This is analagous to human gastric cancer which occurs more frequently in the older age groups.

The induction periods varied somewhat for each tumour type however.

The importance of the mean induction periods of the various tumour types should not be stressed for there are several factors which make its interpretation difficult. The inherent defect in the thread implantation method is that the carcinogen is not exposed in a constant concentration and for a similar period of time in all animals. Therefore, unless the number of animals in each series is large, it is impossible to compare their induction periods. Also in this study the values given for induction periods is the time from the first thread implantation to the time of sacrifice, not to the time of actual development of malignant cells or the development of palpable tumours. Accurate induction period can only be obtained in studies in which the animals are killed at specified intervals, in which case microscopic tumours will be found. In the present study the animals

were kept alive as long as possible, rather than being killed when a tumour was first felt.

For this reason, the carcinomas which grew more slowly than the sarcomas did not kill the rat as quickly after the tumour induction as did the sarcomas. The given induction periods for the carcinomas then are artificially long.

The lowest survival period for a carcinoma was 163 days, this is considerably less than the 9 months (270 days) which Hare et al (167) reported. However in the mouse Stewart, Snell & Hare (162) found a microscopic adenocarcinoma at 42 days.

Although there was a variation in the mean survival times between the various tumour types, the variation was not great and because of the uncontrolled variables mentioned they are not amenable to statistical interpretation.

The presence of the threads at autopsy indicates that there was a slightly greater tendency for the threads to be associated with the tumours than with normal stomachs. Also the earlier series although not surviving as long as the B_{12} series did not have as many threads present at autopsy. The presence of the threads microscopically is not of great significance for whenever possible the threads were

removed before sectioning, also in the larger tumours the sections did not always include the area which contained the thread.

The successful induction of carcinomas in these animals reaffirms the principle regarding experimental gastric carcinoma which have been evident in previous studies. These principles are that the mouse or rat will not develop malignant tumours of the glandular stomach from carcinogens exposed only to the superficial mucosa. This holds true for abnormal mucosa also, hyperplastic or ulcerated mucosa is not susceptible to oral carcinogenesis. However if the carcinogen is exposed to the submucosa of the stomach, carcinogenesis will occur.

B. Criteria of Malignancy.

The problem of the criteria of malignancy has been discussed previously in this paper, when it was emphasized that there are no criteria which are unfailingly accurate. In this study the demonstration of invasion has been considered to be of prime importance. All lesions classified as malignant showed invasion or infiltration of the surrounding structures. Although Stewart (119) is of the opinion that the invasion must extend into the serosa, this restriction is

excessive, for the small tumours obviously may not have reached the serosa simply because they have not grown large enough. Also tumours are seen which invade mucosa, muscle and fat and yet which do not extend to the serosa. As a further point the diverticula although not malignant can penetrate into the serosa, therefore serosal involvement alone is not diagnostic of malignancy.

It is difficult at times to determine whether or not the tumour is actually invading surrounding organs or just pushing against them. In the rat peritoneal irritation as caused by a simple laparotomy, will give rise to close adherence of the abdominal organs. Microscopically it is often difficult to identify the capsule of such organs as the spleen, liver and pancreas. Therefore true invasion must demonstrate strands & islands of tumour tissue extending into the involved organs and not just lying next to the adjacent organ.

Invasion of the lymphatics and blood vessels was almost nonexistent occurring in 1 tumour only, this is in contrast to the reports of others (167). The failure to invade lymphatics & blood vessels may be related to the lack of distant metastases.

As regards the cellular aspects of malignancy, experience with human tumours must serve as a guide. However in the rat, inflammatory processes can give rise to anaplastic changes and consequently in the

assessment of a tumour, one must always look for areas that are free of inflammation. Chronic inflammation in the rat at times appears identical to cellular changes which in the human would be called a fibrosarcoma. Therefore in the rat, less importance must be attached than in the human, to the cellular detail in the assessment of malignancy.

In the tumours diagnosed as malignant, there were always good examples of cellular anaplasia. The sarcomas in particular tended to show loss of polarity, while the adenocarcinomas usually showed less evidence of it.

The failure to metastasize is not infrequent in animal tumours. Regarding the species variation in the biological behaviour of malignant tumours, the human appears to be the most susceptible to the development of metastases. Malignant tumours in the rat typically do not metastasize widely. Therefore it is not unusual that malignant gastric tumours such as these have not metastasized. The peritoneal seeding of sarcomas which occurred in several of the animals in this study may have been peritoneal seedings of the gastric sarcomas. However they may also have been separate primaries induced by the thread which was loose in the abdominal cavity.

Threads were present grossly in only 30% of all animals at autopsy and in 30% of the adenocarcinomas microscopically. Therefore although the tumour is dependent upon the carcinogen for its induction, following induction the tumour can then survive independently of it.

Although the control series was small, the complete absence of malignancy in this group and the lack of spontaneous gastric tumours in this strain of rat are sufficient evidence that the malignancies were due to the carcinogenic properties of the 20-methylcholanthrene.

C. Significance of Diverticula.

The adenomatous diverticula are of particular interest, for they are not dependent on the carcinogen for their induction, yet they do appear to have a role in the development of the carcinomas. They are specific for the rat (62) and do not resemble any type of pathology seen in the human stomach.

Previous studies on the histogenesis of this lesion (152, 170) indicate that the thread ulcerates the mucosa, forming a furrow along the thread. This furrow becomes lined with glandular epithelium and the margins become hypertrophied. If a break forms in the

muscularis the glandular cells soon extend through it. These changes occur with normal cotton threads or with the carcinogenic threads. It is therefore the stimulus of the foreign cotton thread and not necessarily the carcinogen which initiates their development.

Stewart (119, 162) has called the diverticula "precancerous", however their occurrence in the control series indicates that some qualification of the term is required. Grant and Ivy (170) suggest that the lesion is precancerous in the sense that "in their absence the observed invasive and malignant types of epithelial growth would not have occurred and that the probability of a malignant neoplasm arising from such lesions is greater than from normal mucosa". When qualified in this manner the diverticula can be considered to be precancerous.

The precancerous nature is demonstrated by the diverticula in which carcinomas are to be seen arising. The explanation for this undoubtedly is that the diverticula bring the glandular epithelium into the deeper layers in which the carcinogen is lying. Here the carcinogen and glandular cells remain together undisturbed by gastric secretions. Therefore the diverticula are probably not essential for the induction of gastric carcinomas, but they are an important factor in many cases.

The diverticula are undoubtedly not related to the genesis of the sarcomas.

D. Differentiation between Malignant and Nonmalignant Lesions.

The differentiation between the well differentiated adenocarcinomas which are malignant and the diverticula which are benign is difficult. When inflamed, the epithelium of the diverticula appeared atypical, the diverticula with many small lumina in particular, closely resembling the well differentiated adenocarcinomas. Differentiation between these lesions was based upon several points. The glandular spaces of the well differentiated adenocarcinomas, when seen under low power appeared much more irregular in size and shape and were more closely packed than were the diverticula. Also the adenocarcinomas exhibited more cellular atypicality than did the diverticula although the diverticula were often atypical in inflammed areas. The third and most important point was that in the adenocarcinomas, glandular spaces could be found lying with little or no stroma in the tissue spaces beyond the main tumour mass. That is there was evidence of infiltration whereas the diverticula were always limited by a thin collagenous sheath.

E. Carcinomas and Squamous Metaplasia.

The types of carcinomas produced in the rat were similar to those which have been described previously (152, 167, 170), and also in the mouse (162). However lymphatic invasion was not a prominent feature and distant metastases were not seen as has been reported (167).

The presence of squamous metaplasia both in the diverticula as a benign type and in the adenoacanthoma as a malignant type has been a common finding. Squamous metaplasia also occurs in the mouse (162). The squamous elements appear to arise by a process of metaplasia from the glandular epithelium. There is good evidence that it did not arise from the forestomach for in no cases could a connection with the forestomach be demonstrated. Also no pure squamous cell lesions were present, but all of these had some glandular elements, suggesting that the squamous elements arose from the glandular elements and not vice versa. Hare et al (167) who injected the carcinogen in 2 sites, adjacent to the antrum and adjacent to the limiting ridge, found that squamous metaplasia was commoner in the antral lesions. This corresponds to the human where although adenoacanthomas are extremely rare, when occurring

they usually do so in the prepyloric region (152). In contrast to the human stomach the rats stomach appears to be particularly susceptible to squamous metaplasia.

F. Sarcomas.

The sarcomas were arbitrarily classified into 3 types only, this classification being based on experience with human tumours. Those classified as spindle cell sarcomas were probably in some cases fibrosarcomas and occasionally leiomyosarcomas. However no tumours, in spite of the use of van Gieson's stain, trichrome and silver stains, showed any elements suggesting a malignant Schwannoma, as has been reported by others (188).

The 3 types of sarcomas frequently contained elements of the other sarcomas in the same tumour. The anaplastic elements in particular frequently blending with spindle cell tumours. However no carcinosarcomas or tumours with mixed elements of malignant epithelial and connective tissue was seen as has been reported (167).

Since it is impossible to expose the carcinogen only to the glandular tissue and not to the connective tissue, the occurrence of

sarcomas is to be expected. It would be preferable to have a technique which produced carcinomas only and not sarcomas, however this appears to be impossible at present. Since this study was designed primarily to induce and study the adenocarcinomas, the sarcomas have not been studied in as great detail as the carcinomas.

G. Bone Formation.

The presence of ossified bone is an example, like the squamous cells, of metaplasia. It has been observed previously in similar studies (167) in gastric mucosa painted with carcinogens which did not develop malignancies (155) and when subcutaneous tissues are exposed to carcinogens, (189).

The various diets did not appear to affect significantly the rate of bone formation although 50% of that occurring in the second B_{12} series was present in a malignant form.

The absence of any bone in the control series is not strong evidence that a carcinogen is required for its production; as the size of the control series is too small. In 25 specimens 4 cases of bone only would be expected from the ratio produced in the second B_{12} series. A much higher percentage of bone would have been found

in the last B_{12} series if all of each stomach had been sectioned. For the bone is often found around diverticula or in the submucosa. In the specimens with tumours, frequently only the tumour and a small portion of the mucosa would be sectioned.

H. Histogenesis.

The histogenesis of the malignant tumours and the diverticula cannot be properly evaluated from the material available. There were very few sections which illustrated the early changes. It is not possible to state for instance that the adenocarcinomas tended to become more anaplastic with age and further exposure to the carcinogen. Also it is not known whether the anaplastic tumours arise as such or whether they arise as more differentiated tumours.

I. Effect of Modifying Factors.

A great deal of significance cannot be given to the series in which various drugs and diets were used. There are too many uncontrolled variables and also it is doubtful if the animals on deficiency diets were actually deficient. Except in the last B_{12}

series there is no objective evidence that the diets and drugs were given in sufficient dosage to cause the desired metabolic effects. The modification of the thread implantation method midway in the experiment makes it impossible to compare the later series with the early ones.

A method of introducing a carcinogen such as this thread implantation method has the inherent disadvantage that the carcinogen is not exposed to the tissue in all animals for the same period of time and in the same concentration. For the thread sloughs from the stomach at variable periods, reinsertion does not always replace it in exactly the same location and reinsertion is technically impossible in 10% of animals due to adhesion formation.

J. Effect of Drugs.

The cortisone and Lithospermum series were designed to evaluate the effect of endocrine abnormalities on tumour induction. Although cancer of the stomach in humans is not an endocrine dependent tumour in the manner of breast and prostate carcinoma, there is a marked sex variation.

The ingestion of the ground up root of the plant Lithospermum

ruderale or parentered extracts of this plant has been shown to cause gonadal atrophy, adrenal cortical hyperplasia and atrophy of the thyroid in the rat (190, 191, 192). Although the active substance has not been isolated as yet, it appears to antagonize the hypophyseal gonadotrophins, thyrotropic hormone and a concomitant stress reaction gives rise to increased adrenal corticosteroid secretion.

Although only 15% by weight in the diet has been shown to produce the desired effect (191), and these animals were fed 20% by weight, no estimation of gonadal atrophy was done in these animals and it can only be presumed that the animals were ingesting sufficient quantities.

Although the Lithospermum did not significantly affect the tumour induction, there was a significant number of gastric ulcers in this group. This ulceration may have been due to endocrine imbalance, such as excess corticosteroids, or to the direct irritating effect of the root in the diet. Another possible explanation is that the anorexia caused by the diet may have produced nutritional deficiencies such as a vitamin A deficiency. This group of rats did not gain weight as rapidly as did the other groups.

There were no abnormal findings such as gastric atrophy, ulceration or varieties in tumour induction found in the animals injected with enterogastrone. This hormone which is released by the upper small intestine inhibits the motor and secretory activity of the stomach (193).

The gastric washings with alcohol also produced no significant findings. The lack of gastritis and ulceration indicate that the alcohol in the dosage used did not affect the gastric mucosa.

K. Effect of Dietary Deficiencies.

The groups fed the vitamin A deficient diet produced no significant findings. These animals were probably not actually deficient for there were no gastric mucosal changes as would be expected with vitamin A deficiency.

Because of the association in the human between pernicious anaemia and gastric cancer, it was felt that an investigation of the effects of intrinsic factor and vitamin B_{12} on the tumour induction was warranted. The study with intrinsic factor, although done on a small group of animals only indicated that this substance did not radically alter the tumour induction rate from the vitamin B_{12} series,

in which the same method of thread insertion was used.

The initial vitamin B_{12} series were done with the earlier method of thread insertions and the rate of tumour induction was very low. Therefore the analysis will be limited to the second group. Regarding this group it must be realized that the deficient group was not in actual fact truly deficient. The serum vitamin B_{12} estimations indicate that the serum levels although in the low normal range were not in the true deficiency range (194). The rat stores vitamin B_{12} in the liver and kidneys and it is most difficult to create a deficient animal (195, 196, 197).

It must be emphasized when comparing the results in the supplemental and deficiency groups that there are certain uncontrolled variables which make statistical analysis difficult. These variables were mentioned earlier in the discussion, the one of greatest significance here is that the deficiency group survived somewhat longer than the supplemental group, the mean survival time in the deficiency group was 400 days compared to 360 days in the supplemental group. As a greater number of tumours occurred in the deficiency group, the significance of this greater incidence is increased. However in the following analysis this factor has been

ignored.

A comparison of the tumour induction rate between the 2 groups indicates that it is significant that more tumours occurred in the deficiency series than in the supplemental and also it is significant that more adenocarcinomas occurred in the deficiency series. The variation in incidence of the other tumour types is not significant. It is presumed that this variation is due to the low vitamin B_{12} rather than the excess, although the opposite could be the case.

The degree of significance has been determined by the method of chi square, fourfold tables and probabilities of chi square (198).

The determinations are as follows:

Variation in incidence of all tumour types in the vitamin B_{12} deficient group and the vitamin B_{12} supplemental group:

> Chi square - 4.05
> Plies between 0.05 and 0.01 and the variation is moderately significant

Variation in incidence of adenocarcinomas:

> Chi square - 4.35
> Plies between 0.05 and 0.01 and the variation is moderately significant.

Variation in incidence of Adenoacanthomas:

> Chi square - 0.858
> Is greater than 0.10 and the variation is not significant.

Variation in incidence of Spindle Cell Sarcomas:

Chi square - 0.104
is greater than 0.10 and the variation is not significant.

Variation in incidence of Osteogenic Sarcomas:

Chi square - 2.02
is greater than 0.10 and the variation is not significant.

Variation in incidence of Anaplastic Sarcomas:

Chi square - 0.066
is greater than 0.10 and the variation is not significant.

It is of interest that in the earlier vitamin B_{12} series that 4 tumours developed in 22 animals in the deficiency group and only 3 tumours in 31 animals in the supplemental group.

The relationship of this finding of an increased incidence of adenocarcinomas in the vitamin B_{12} group to human gastric cancer and pernicious anaemia is at best theoretical. The primary defect in pernicious anaemia is in the stomach and is characterized by gastric atrophy and failure to produce sufficient intrinsic factor (199). The deficiency in vitamin B_{12} is secondary to this.

Since gastric atrophy unrelated to pernicious anaemia has an increased incidence of gastric carcinoma, it would seem that the relationship between pernicious anaemia and gastric carcinoma would

be due to the gastric atrophy rather than the deficiency in vitamin B_{12}. This would seem to be the case particularly because this increased tendency of patients with pernicious anaemia to develop gastric carcinoma occurs in treated cases which have an adequate quantity of vitamin B_{12}.

The significance of this variation in incidence with vitamin B_{12} is not great, it would be of interest to implant threads in a large group of animals truly deficient in vitamin B_{12}.

L. Multiple Tumours.

The occurrence of multiple tumours or combinations of separate tumours in the same animal is to be expected, the number occurring in this study actually somewhat less than the statistically expected number. The calculated expected number can be determined with this formula:

$$\frac{\text{specimens of tumour A}}{\text{total number of specimens}} \times \frac{\text{specimens of tumour B}}{\text{Total number of spec.}} \times \text{Total number of specimens}$$

Tumour Combinations	Calculated #	Actual #
Adenocarcinoma with spindle cell sarcoma	3.3	1
Adenocarcinoma with osteogenic sarcoma	1.4	2
Adenocarcinoma with anaplastic "	2.1	-
Adenoacanthoma with spindle cell "	2.6	3
" " with osteogenic "	1.1	1
" " with anaplastic "	1.6	1
Osteogenic sarcoma with " "	0.81	3
Spindle cell " with " "	2.1	2
Total (series prior to B_{12})	2.4	-
Total (all series)	17.5	13

All the combined tumours were examples of separate tumours and not combinations of different tumour types within the same tumour. It was not possible to differentiate the adenocarcinomas and acanthomas into separate tumours, as they normally occurred in combinations.

M. Transplants.

Although none of the carcinomas survived as transplants, this is not to be taken as conclusive evidence that the tumours were not

malignant, for ability to survive transplantation is not required as an absolute criteria of malignancy. In the mouse successful transplantation of carcinomas has been done (161). The anaplastic sarcomas microscopically appeared more malignant and biologically behaved more so. Therefore it is not surprising that 2 of the 4 transplanted survived.

N. Spontaneous Tumours.

It is impossible to state whether or not the reticulum cell sarcoma occurring in the lung was related to the intragastric carcinogen. Since in this animal there were no malignant lesions in the abdominal cavity, it is unlikely that it was a metastasis. There is also no evidence that the carcinogen was carried by the blood or lymph to the lungs, if it could be absorbed in this manner more distant tumours would be expected.

The chief cause of natural death in this strain of rat is pulmonary infection with abscess formation. The abscesses are white, firm and grissly; it is impossible to differentiate them from tumours. Therefore although pulmonary tumours have not been previously described in this strain of rat, they could be relatively common and

yet pass unnoticed.

O. Analogies to Human Cancer.

The production of gastric adenocarcinoma in the rat by the method of carcinogen implantation has very few analogies to the problem of human gastric cancer. Certainly there is no evidence that chemical carcinogens are ever lodged in the submucosa of the human stomach. However now that a method is available which will produce carcinomas in 25% of animals it will be possible to investigate systemic factors affecting carcinogenesis. The present study with vitamin B_{12} indicates the possibilities which there are. Since the human stomach has no comparable lesions to the adenomatous diverticula which were seen, the mechanism of gastric carcinogenesis in the human would appear to be different from that in the rat.

VII

SUMMARY AND CONCLUSIONS.

1. The present work has dealt with the induction of adenocarcinoma of the glandular stomach in the rat, with the carcinogen 20-methylcholanthrene. The implantation of cotton threads saturated with this compound, in the submucosa of the glandular stomach of the rat, has produced adenocarcinomas of the stomach in a significant number of animals.

2. A review of the factors known to affect the incidence of cancer of the stomach in man has been presented. The problem of experimental gastric carcinogenesis has been reviewed with particular reference to the rat and to the use of implanted carcinogens.

3. A method of tumour induction is presented which has resulted in a significantly higher incidence of tumours than has been previously reported. This method of thread implantation involves the insertion of a cotton thread saturated with 20-methylcholanthrene in the submucosa, along the greater curvature of the stomach. The thread is fixed in position with 3 silk stay sutures. The animal is

then reoperated upon at 3 and 6 month intervals and new thread inserted in a similar manner.

4. The malignant tumours produced included adenocarcinomas, adenoacanthomas, spindle cell sarcomas, osteogenic sarcomas and anaplastic sarcomas. The number of carcinomas arising in the series with the described method of thread implantation was 43 tumours in 173 animals. A benign adenomatous diverticulum is also produced in 70% of animals, this lesion is dependent for its development on the presence of the cotton thread and not on the carcinogen. This lesion is precancerous.

5. The effect on tumour induction of various diets and drugs has been studied utilizing this method of tumour induction. The rate of tumour induction and specifically the rate of production of adenocarcinomas was significantly higher in animals partially depleted in vitamin B_{12} than in animals receiving supplemental vitamin B_{12}.

6. The induction period for the malignant gastric tumours is relatively long, approximately one-third the life span of a rat.

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