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EXPERIMENTAL PRODUCTION OF GASTRIC NEOPLASMS

IN THE RAT

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EXPERIMENTAL PRODUCTION OF GASTRIC NEOPLASMS IN THE RAT

by

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PREFACE

This work embodies the results of an investigation done under the supervision of and in collaboration with Dr. Donald R. Webster, Director of the Department of Experimental Surgery, to whom I am deeply indebted for his guidance and assistance.

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CHAPTER I

INTRODUCTION

I THE PROBLEM OF CLINICAL GASTRIC CANCER

A. STATISTICS

Cancer of the stomach is the most frequent of all malignant growths. Livingston and Pack in 1939 (121) have stated: "There are more deaths from cancer of the stomach than from all malignant tumors of lip, tongue, cheek, tonsil, pharynx, larynx, salivary glands, thyroid, male and female breast, ovary, uterine cervix and corpus uteri combined." (121, p. 1) Wangensteen in 1947 (231) reported that of the 150,000 annual deaths from cancer in the United States, approximately 40,000 die of gastric cancer.

The onset of gastric cancer is insidious, and, from the recent mass x-ray surveys of the stomach reported by Morgan in 1950 (140), it is concluded that a much longer asymptomatic phase exists than was formerly suspected. This preliminary phase probably lasts three

years before the first mild symptoms of indigestion occur. From this, it is easy to see why the patient fails to seek medical aid early, and why the physician fails to diagnose the disease early. By the time the patients reach the hands of even the most experienced surgeon, approximately half of the total number of cases are too late for suitable resection (205, 225). If an "adequate" resection can be done, which many surgeons (75, 114, 146, 229, 83, 122, 199) now believe should be a total gastrectomy with regional lymph node dissection, 20 to 30 per cent of the patients will survive five years or more (230).

However, the best five year survival rate of an entire group of patients entering a hospital with the diagnosis of gastric cancer is still only 7 per cent (233). These are depressing figures, and the only method available now to improve these figures is to reduce the delay period. This is being attempted by various means:

1. Education and examination of the general public in Cancer Detection Clinics (205).
2. Education of the general practitioner by "refresher" courses in cancer diagnosis (34).
3. Mass gastric x-ray surveys of the general population (140, 218).
4. X-ray follow-ups of special groups, such as, all patients with pernicious anemia (206).

B. CONCEPTS OF THE CAUSE OF GASTRIC CANCER

Two basic concepts of the cause of gastric cancer have influenced the clinical and experimental approach. The concept that some substance ingested by the organism leads to the formation of gastric cancer has motivated many experiments. These extrinsic factors have been studied in both man and animals, but no conclusive evidence has yet appeared.

The second concept that some condition or substance intrinsic to the organism itself leads to the

formation of gastric cancer has also formed the basis of extensive experimental work. Many of these intrinsic factors have also been investigated in man and animals, but no definite conclusion can yet be drawn concerning their influence.

To simplify the discussion of the literature on gastric cancer these two concepts will be treated separately, but with the understanding that it is often difficult or impossible to separate them either clinically or experimentally.

C. EXTRINSIC FACTORS - DIET AND GASTRIC IRRITANTS

The fact that man is the only animal to eat hot and highly spiced food led Lerche (118), in 1916, to study the regions of the stomach most often burned by swallowing corrosive fluids accidentally or for suicidal purposes. He found that cicatrices occurred most often in the cardia, along the "gastric gullet", and in the prepyloric region; areas in which 79 per cent of cancer of the stomach are found. It appears significant that the favorite sites of

carcinoma of the stomach correspond exactly to the sites of cicatrix from corrosive fluids. The explanation of the localization of the burns to these areas of the stomach lies, of course, in the mechanics of swallowing. Therefore, hot food or irritating liquids were considered as possible causes of carcinoma of the stomach.

Since recent statistics have shown that carcinoma of the stomach is two and a half times as common in Holland as it is in England, in spite of the fact that cancer as a whole has the same incidence in both countries, Lintott (120) and Herbert (87) at Guy's Hospital, London, tried to find the reason for this. In 1936, Lintott reported the results of routine test meals given to over 500 English patients and compared the gastric acidities with those found in a similar group of Dutch patients in Holland. There was no significant difference in gastric acidities between the two groups. From this, he concluded that the evidence was in favor of an extrinsic factor. In these same two groups of patients, Herbert (87) investigated the type of diet, masticatory efficiency, irritation from swallowed dental

sepsis, thermal irritants, and chemical irritants, such as condiments, alcohol, and tobacco. He found that the difference in diet consisted of a higher consumption of bread, cheese, and vegetables, and a lower consumption of meat among the Dutch. The incidence of open oral sepsis, the temperature of the food taken, and the consumption of spiced foods, spirits, and tobacco were considerably higher for the Dutch group.

In 1938, Bonne (25) published studies done over a long period in Java where he had the unusual opportunity of studying two different racial groups, each having a marked difference in the incidence of gastric carcinoma. The Malays in Java have a very low incidence of gastric cancer. Only one gastric carcinoma was found in 3, 885 autopsies over a 15 year period, although the total cancer rate was in accord with the usual figures for western countries; whereas, in the Chinese who had immigrated to Java, the incidence of gastric cancer was the same high rate as for western countries. Gastric morphology and physiology were studied in the two groups, as well as their dietary habits,

but no marked differences were found. Bonne and his co-workers were unable to explain the disparity in incidence of gastric cancer in these two groups.

Pack and McNeer (148), in 1948, reviewed the literature on the relation of diet to the incidence of gastric cancer. They found that gastric cancer is relatively infrequent in Mexico where hot tamales and chili are dietary staples. It is a common malignancy in Persia where meat eating is usual, but unknown among Eskimos who eat large quantities of meat. Pack and McNeer conclude, that on the basis of our present knowledge, diet offers few clues to the cause of gastric cancer.

D. INTRINSIC FACTORS

1. Aging

Although cancer of the stomach is by no means confined to the upper age brackets, it is primarily a disease of middle and late life (148), occurring most frequently between the fiftieth and seventieth years. Pack and McNeer state that: "This age factor is highly significant, pre-

disposing to a greater incidence wherever life expectancy exceeds the fifth decade." (148, p. 522) For example, in 1940, 20.4 per cent of the population in the United States were over 50 years of age, and the death rate from gastric cancer was 19.8 per 100,000. The progressive senile atrophy of the gastric mucosa with its hypo-function and anacidity, as described by Popoff (161), may well be one of the intrinsic factors in gastric cancer.

2. Heredity

The difficulty of obtaining reliable family histories has hindered studies on the repeated occurrence of cancer in certain families (198). Schinz (172), after reviewing many case histories and the extensive literature, concluded that there is no proof that a predisposition to malignant disease is inherited in man. Wangensteen (230) was able to obtain a family history of gastric cancer in 15.6 per cent of the 200 patients with gastric cancer questioned in his clinic. However, it is impossible at this time to draw any conclusions concerning the factor of heredity in gastric cancer.

3. Endocrine

Endocrine factors in the production of carcinoma of the breast have been extensively studied, and evidence exists to show that sex hormones can produce breast cancer (131). No work, however, can be found to show that any of the known hormones influence the occurrence of gastric cancer.

4. Neurofunctional

Lesions in the interbrain were shown to have a direct effect on gastric mucosa by Cushing in 1932 (46). Experiments based on Cushing's work will be discussed later.

The fact that gastric ulcers can arise on a neurofunctional basis (161), and that cancer of the stomach has been found in chronic gastric ulcers (112, 64, 2) has led to speculation, but as yet to no conclusions on the neurofunctional factor in gastric cancer.

5. Secretory

Wide variation in gastric secretory activity may occur in people free of disease (161), but a dimished secretion of hydrochloric acid is a frequent finding in patients with gastric cancer (182). It is impossible to determine whether

this achlorhydria is due to a concomitant anemia (7), or whether it is due to the secretory depressant found by Brunschwig (30) in achlorhydric carcinomatous stomachs. Wangensteen (230) and his co-workers (206) believe that achlorhydria may be one of the precursors of gastric cancer and have undertaken a careful follow-up study by means of repeated x-rays, gastroscopic examinations, and fractional gastric analyses with histamine on all patients with achlorhydria admitted to the clinics of the University of Minnesota Hospitals.

6. Metabolic

Metabolic abnormalities in patients with gastric cancer have been studied by Abels et al. (1) at the Memorial Hospital for the Treatment of Cancer and Allied Diseases. They concluded that the removal of the cancer is usually followed by a disappearance of the metabolic dyscrasias.

Substances have been extracted from human tissues which are carcinogenic when injected into experimental animals. These exist in cancerous and non-cancerous patients. Since these substances have been used only experimentally, they will

be discussed in detail later.

Some of the intrinsic factors just mentioned no doubt play an important role in the problem of gastric cancer, but, until further information is obtained, no conclusions can be made.

Although there is a wide diversity of opinion concerning the origins of gastric cancer, most authorities (170, 112, 64, 164, 171, 182, 231, 2, 134, 230, 85, 43, 109) agree that it does not arise de novo in normal gastric mucosa. Gastric polyps, atrophic gastritis, and gastric ulcers have all been known to precede malignant change. It is not easy, however, to follow malignant development in gastric mucosa clinically, even by the latest technics of gastric cytologic studies (149, 31). It is for this reason that the production of gastric cancer in experimental animals is of such great importance (108, 99, 153).

II THE PROBLEM OF EXPERIMENTAL GASTRIC CANCER

The production of experimental cancers in various tissues of many experimental animals is generally believed to have started with Yamagiwa and Ichikawa's discovery, in 1914 (242), that repeated applications of coal tar to a rabbit's ear were followed in some cases by carcinomatous changes with metastases. Yet the production of experimental cancer in the glandular stomach of any animal is still extremely difficult, and, in many species, has been impossible to obtain up to the present time. In view of the rapid strides that have been made in the general field of experimental cancer (173), it is of great importance to review the efforts made and the reasons for the lack of success in producing experimental gastric cancer.

A. SPONTANEOUS GASTRIC CANCER IN ANIMALS

Although gastric cancer is one of the most common malignant growths found in man (121), it is one of the rarest spontaneous neoplasms of animals (108, 196, 234).

Slye, Holmes, and Wells reviewed the literature on spontaneous malignant neoplasms in wild and domestic animals

in 1917 (196) and brought this review up to date in 1938 (234). Gastric neoplasms are so rare that they are reported as single cases. For example, 381 baboons were examined, and only 1 gastric cancer was found. Dr. L. E. Day, in a personal communication to these authors, stated that in the enormous amount of material he had observed at the Chicago Stock Yards, there were no examples of gastric carcinoma. Laboratory animals have been studied extensively in search of spontaneous gastric neoplasms with the object of finding a "susceptible" strain from which to breed laboratory animals with spontaneous gastric cancers (108). To be comparable with gastric cancer in man, the neoplasms must be in the glandular stomach, not in the rumen, or forestomach, which is covered by squamous epithelium (108).

McCoy (132), in 1909, examined the gastrointestinal tract of 100,000 rats without finding a single neoplasm. Bullock, Curtis, and Dunning, in 1930 (33), and, in 1931 (45), reported one gastric cancer in 33,000 rats examined postmortem. In 1946, 15,625 rats from two inbred lines were

examined post mortem by Dunning and Curtis (56). They found 3 small gastric adenocarcinomas without metastases (57). Ratcliffe (163) reported from the Wistar Institute in 1940, that no gastric cancers were found post mortem over a 5 year period in two colonies of rats having a yearly census of over 2000. In 1950, Olcott (147), using Sherman-Mendel rats, found 10 spontaneous neoplasms in 500 autopsies. No tumors occurred in the stomach. The stomachs of 400 rats of the Royal Victoria Hospital strain have been examined during the last 2 years for this work, but no tumors have been discovered.

Mice develop more spontaneous tumors than rats, but the number of adenocarcinomas of the stomach is still extremely small. In over 142,000 mice of the Slye stock, dying of natural causes, Wells, Slye, and Holmes (234) found only 3 adenocarcinomas of the stomach. These represent all the gastric cancers found in this stock over a 20 year period of careful observation. In papers published in 1943, 1945, and 1948, Strong (223, 221, 222) has reported that he has bred a gastric cancer-susceptible

strain of mice by injecting a carcinogen into five generations of mice to start off this strain. Andervont (5), who has worked extensively with this strain, stated in 1949 that: "The lesion is not considered to be malignant, because it develops symmetrically, conforms to a general pattern in all mice, and is not generally transplantable. Thus far, metastases have not been seen". (5, p. 405)

The conservative opinions of Andervont and his group (102) at the National Cancer Institute in Bethesda are valued highly in experimental gastric cancer research. Therefore, until more evidence is given, it would be wise to reserve judgment on the production of a gastric cancer-susceptible strain of mice.

This marked difference in occurrence of spontaneous gastric neoplasms between man and lower animals has never been explained satisfactorily, although it has formed the basis of many experiments.

B. CRITERIA FOR THE EVALUATION OF EXPERIMENTAL GASTRIC CANCER

A vast literature has been published on the results of experimental gastric carcinogenesis. Unfortunately this has not always been pertinent to the subject. For example, squamous cell cancer of the forestomach, in animals with a rumen, is not analogous to adenocarcinoma of the secreting glandular stomach in man (108), and erroneous conclusion can be drawn if this is not kept constantly in mind. A more serious mistake in judgment is made when any neoplasm of the stomach is called a cancer.

In order to clear up the confusion which existed on these points in the early literature and to establish criteria for the evaluation of future work, Klein and Palmer (108) made a critical review of the entire literature on experimental gastric cancer in 1941 and established the following criteria:

"Induced cancers should have those characteristics generally considered inherent in a malignant growth:

1. the ability to proliferate independently as metastases;
2. the ability to invade progressively and destructively neighboring tissues and organs;
3. irreversibility of these properties in the absence of the extrinsic factor initially held responsible for the cellular change;
4. reasonable evidence to indicate a causal relation of the experimental procedure to the tumor

Application of these criteria to induced gastric tumors is not always easy, but unless they can be demonstrated, the malignant nature of the tumor cannot be considered as proved. The histologic appearance of the tumor alone is not adequate." (108, p. 582)

C. EXTRINSIC FACTORS-DIET AND GASTRIC IRRITANTS

Many experiments have been carried out to explore the effect of diet on the gastric mucosa. Those experiments which produced lesions only in the forestomach of animals have been omitted here.

In order to reproduce the irritating effects of coarse foods, Bullock and Rohdenburg (32), in 1918, introduced celluloid balls covered with pig's bristles through a gastrotomy in the rat's forestomach. After a few weeks, polypoid growths appeared in the glandular stomach with inflammation in the gastric wall. Other irritating materials, such as scarlet red powder on a rubber sponge, also produced lesions in the glandular stomachs, but, although these lesions were similar on microscopic study to benign gastric cystadenomatous lesions occasionally reported in man (76, 174), no malignancies developed. They concluded, that irritation alone is an insufficient factor.

In 1947, Dyer, Kelly, and Dunn (59) administered repeatedly such irritating liquids as hot water, hydrochloric acid, lactic acid and sodium hydroxide in various strengths by stomach tube onto the glandular mucosa of mice. They obtained no malignancies and concluded: "That the stomachs showed so little injury following very drastic treatment is evidence of the remarkable

resistance and recuperative power of the normal gastric mucosa." (59, p. 70)

Davis and Ivy (48), in 1949, after reviewing the literature concerning heat in gastric carcinogenesis, felt that hot food may be concerned in the etiology of peptic ulcer, chronic gastritis, and carcinoma of the stomach. By feeding hot mush to dogs, they determined the time and temperatures necessary to cause thermal injury to the gastric mucosa of dogs, but have not, as yet, carried on the experiments for a sufficient length of time to show chronic changes in the gastric mucosa which might lead to gastric malignancy. According to Berenblum (16), however, there is little danger that an ordinary irritant would produce a tumor of its own accord, but, if a "preneoplastic lesion", such as a chronic ulcer, could be produced by hot foods, then, he felt, its progress to cancer might be hastened by the action of a variety of nonspecific irritants.

The temperature of the food itself, when ingested, may not be of nearly so much importance in gastric carcinogenesis, however, as the changes caused in fats by cooking

at high temperatures, such as, in the deep fat frying of potato chips, fish, and doughnuts. This took on added importance when it was discovered that at high temperatures cholesterol could produce the potent carcinogenic hydrocarbon, methylcholanthrene (9, 65).

It was very unfortunate that the first experimental work done along these lines was by a scientist whose integrity was later in question, because that led many investigators to ignore this field for several years. In 1938, Roffo (165) reported that he had induced adenocarcinomas in the glandular stomachs of rats by feeding them animal fats or olive oil heated to 350° C. for half an hour. These lesions were not considered true malignancies by Klein and Palmer (108) in their critical review, and their comment on his work was that: "In the absence of any mention of a metastasis from a gastric lesion or of an instance of invasion of a neighboring structure or organ, the evidence for malignancy is not entirely convincing." (108, p. 573) Roffo's work has been repeated by many investigators (19, 105, 106, 107, 117, 91, 153, 15, 154, 157, 155)

and, although ulcers (142) and proliferating glandular elements deeply placed in the submucosa and in the external muscle layer of the stomach were obtained (167), none of the lesions can be classified as a malignancy.

Peacock's work must be especially mentioned, because he has long believed that there is considerable evidence to suggest that gastric cancer is largely due to extrinsic factors, and he has carried out numerous feeding experiments on rats and mice over many years using heated fats and lipoids (153, 15, 154, 157, 155) without obtaining gastric adenocarcinomas. He and his associates (155) at the Royal Cancer Hospital, Glasgow, have consistently failed to identify any known carcinogen in any of the numerous fats tested by their method which allows the detection of 1 part in 100 million of such known carcinogens as 3:4-benzpyrene or methylcholanthrene deliberately added to the heated fat. This work is continuing and may still produce results, but, up to the present time, no true gastric malignancies have been produced by feeding heated fats.

Feeding experiments which simply use the oral route as one means of introducing carcinogenic hydrocarbons into the animal's body are not pertinent to this review of the literature and are not included here. However, one of these potent carcinogenic hydrocarbons, methylcholanthrene, has been prepared from cholesterol (9), which is part of a normal human diet and, for this reason, is of interest. It has been administered orally in many experiments (125, 215, 185, 186), but has not, as yet, produced adenocarcinoma in the glandular stomach of any animal.

Deficiency diets have long been known to produce changes in the stomachs of experimental animals (150), and these changes have been so severe, especially in the forestomachs (92), that they have been reported as malignant (108). It is especially important in all attempts to produce carcinoma of the glandular stomach to recognize that diets deficient in protein (183), vitamin B (224), and other factors as yet unidentified (29), produce ulcers and hyper-

plastic lesions which closely simulate malignancies (97, 141, 18). Therefore, in all experiments designed to investigate carcinogenesis in the glandular stomach of rats, dietary factors must be carefully controlled.

Nettleship (145) has reviewed all the extrinsic factors which might play a role in gastric carcinogenesis and, to complete the list, mentions bacteria, bacterial products, and viruses as possible factors, although experimental evidence is lacking (161).

Finally, in closing the review of the literature on extrinsic factors in experimental gastric cancer, Fibiger's famous work, published in 1913, must be mentioned (68, 69). He believed that he had found the cause of gastric cancer in the parasite Gongylonema neoplasticum. In his experiments, he fed cockroaches, infested with this parasite, to rats and mice and obtained hundreds of tumors of the glandular stomachs. Of all these tumors, only one adenocarcinoma in the glandular stomach of a mouse fulfilled the criteria set up by Klein and Palmer (108). His results have

been repeated by various workers (151, 44, 22) using the same and other parasites, but no other malignancy has ever been reported.

D. INTRINSIC FACTORS

The concept that some condition or substance intrinsic to the organism itself leads to gastric cancer has formed the basis of very little experimental work. Nettleship (145) has enumerated these intrinsic factors as aging, heredity, endocrine, neurofunctional, and secretory. A sixth intrinsic factor, a metabolic factor, has been more recently studied (209, 208, 210).

1. Aging

In people, cancer of the stomach is primarily a disease of middle and late life. It would be appropriate, therefore, to pick animals with similar life spans for work in experimental gastric cancer. Unfortunately, this is usually impossible because of expense. When it has been done, as in Pfeiffer and Allen's (159) experiments extending over 10 years with Rhesus monkeys, no cancers have been obtained. Nettleship (145) believed that a long-lived strain

of dogs would probably be ideal, but as yet no gastric cancers have been obtained in dogs (49). Woglom (239) has stated that: "It is not the age of the body, but the duration of the irritation that is the decisive factor." (239, p. 738) This appears to be true, because the few gastric adenocarcinomas that have been obtained in rats have not occurred until 12 to 14 months after the start of the experiments (35, 212).

2. Heredity

No strain of animal has yet been bred which produces gastric cancer. Although Strong (222) has stated that he has produced a cancer-susceptible strain of mice, Andervont (5) does not believe these lesions are malignant.

3. Endocrine

There are few reports in the literature on experimental gastric cancer concerning the influence of the endocrine glands on the gastric mucosa. Estrogens were used by Pfeiffer and Allen (159) on Rhesus monkeys, but no carcinoma was produced anywhere in the body. Smith and Strong (197), working with mice, found that an adenomatous hyperplasia

near the pylorus occurred with greater frequency among castrated male and female animals and concluded that: "The data indicated that the absence of the sex hormones has influenced the development of the gastric lesion in mice." (197, p. 427) Dodds (53) found that injections of posterior pituitary extract produced an acute ulceration of the gastric mucosa of rabbits, and, in a few cases, a chronic punched out ulcer remained. The volume and acidity of the gastric contents in dogs were decreased by injections of parathyroid extract in Schiffrin's experiments (169).

From all these endocrine investigation, new approaches to experimental work on gastric cancer may be made.

4. Neurofunctional

Cushing's work (46) launched many experiments on the effect of the midbrain on gastric mucosa (145, 93). The results of such experiments are impossible to dissociate from the effect of concurrent injuries to the brain (161). The erosions in the stomach, although often multiple and hemorrhagic, are always confined to the mucosa, and there seems to be little relationship in the results of these experiments to gastric cancer.

5. Secretory

The complexity of gastric physiology becomes more complex in the pathologic state. From achlorhydric carcinomatous stomachs, Brunschwig (30) was able to extract a potent secretory depressant. He demonstrated a similar depressant in the gastric juice of patients with pernicious anemia (28). Other gastric secretory depressants, enterogastrone (101) and urogastrone (81) have been isolated from patients with normal gastric function.

There appear to be, from Babkin's studies (8), some inherent protective mechanisms in the stomach which guard against mucosal irritation. The mucous barrier has been studied in many experiments (77, 99, 11, 95, 204), and Barrett (11), agreeing with Ivy (99), stated that: " The failure of all attempts to induce gastric adenocarcinoma by feeding carcinogenic chemicals to mice and rats is usually explained on the protective barrier role of the gastric mucus." (11, p.143)

6. Metabolic

During the past 10 years, many articles have appeared in the cancer literature suggesting a relationship between bile

acids and carcinogenic hydrocarbons. In 1940, Cook and Kennaway (41) reported that they had induced tumors with deoxycholic acid. Shear (189), however, was unable to obtain the same results. In the laboratory, 20-methylcholanthrene has been prepared from deoxycholic acid (13, 9), but as yet there is no proof that a similar reaction in the metabolism of bile acids takes place in the human body. Substances which are carcinogenic to experimental animals have been extracted from many human cancerous tissues (135, 178, 90, 210). Since 1936, Steiner and his associates (209, 208, 210) have been testing human tissue extracts from cancerous and non-cancerous patients and have repeatedly demonstrated a tumor-inducing factor in the non-saponifiable lipid fraction of human liver from cancer-bearing and non-cancerous persons. Peacock (153) stated that: "The preference of carcinoma for the pyloric end of the stomach, often stopping abruptly at the pyloric valve, is compatible with the conception of the regurgitation through the pylorus of a bile-soluble carcinogen." (153, p. 127) It is for this reason that methylcholanthrene has been used so extensively in attempts to produce gastric cancer in experimental animals.

CHAPTER II

MATERIALS AND METHODS

I OBJECT

From the review of the literature, it can be seen that no satisfactory method is available for producing gastric cancer in experimental animals. The object of this work was to find a consistent means of producing experimental gastric adenocarcinoma. Feeding experiments have the advantage of being physiologic, but, as yet, have not produced true malignancies. Van Prohaska, Brunschwig, and Wilson (227) have pointed out the difficulty of keeping a carcinogenic agent in the stomach, and, even when this has been accomplished, of bringing this agent into intimate contact with gastric mucosa normally covered with mucus. It was felt at the start of this work that if a technic could be devised to expose a portion of the gastric mucosa so that carcinogens could be applied locally, then these two difficulties could be surmounted.

II REVIEW OF PREVIOUS OPERATIONS TO EXPOSE GASTRIC MUCOSA

Gastric mucosa was probably first exposed on the anterior abdominal wall in Pavlov's laboratories for the study of gastric physiology (232). In 1930, Drury, Florey, and Florey (54) made a Thiry fistula from the colon of a dog. This fistula opened up accidentally, so it was used to study vascular reactions of the colonic mucosa to fright. Florey and Harding (77) published a paper on the function of Brunner's glands and the pyloric end of the stomach in 1933. In this paper they stated that: "A 'patch' of fundal stomach was inserted into the abdominal wall by a technique similar in essentials to that of making 'patches' from other parts of the intestine." (77, p. 445) Cats were used for this procedure, but no mention is made of the technic other than the sentence quoted above. In 1945, Kolouch (111) studied chemical and other injuries to exposed mucosal surfaces. He gave no technic, but stated: "When the observation of the late effects of this chemical trauma is to be observed, a perm-

anent pedicled flap graft of intestinal mucosa is employed." (111, p. 641)

One month after the technic, which is described in this thesis, was devised, an article by Miller et al. (139) appeared, in which a similar procedure on dogs was briefly described. They studied the resistance of explanted gastric mucosa to various chemical and physical agents.

In 1949, Dr. Donald R. Webster, Director of the Department of Experimental Surgery, McGill University, developed a technic for making a Heidenhain pouch in rats. Fifty of these operations, with the assistance of Dr. Leon Heller, were done. At the time of operation, methylcholanthrene -cholesterol pellets (187, 188) were inserted into the pouches. The edema of the gastric mucosa often caused these pellets to be extruded. In other animals, a "foreign body" reaction occurred, and both the pellet and pouch were lost by the development of an ulcer in the skin over the pouch.

The operative technic, developed by Dr. Webster, was used at the beginning of this work, but the methylchol-

anthrene-cholesterol pellets were not inserted until 10 days after the operation, when the post-operative edema of the mucosa had subsided. After 36 operations were done, it was decided to abandon this procedure, for the following reasons:

1. The time necessary to complete one operation was one and a half hours. This precluded its use in setting up an experiment requiring a large number of operated animals.
2. No animals with intact pouches containing methylcholanthrene-cholesterol pellets remained after 8 months of operating.

III THE EXPERIMENTAL ANIMAL

The animals best suited for experiments attempting to produce gastric cancer would naturally be monkeys or dogs, because of their longer life span. It was impossible, however, to use such animals for this work, because of the expense involved in buying and maintaining sufficiently large numbers. Of the smaller animals used for experimental purposes, rats seemed to be the animals

best suited for this work, since Ivy (100) has stated that: "When a satisfactory method is available for consistently producing gastric cancer in the rat, we shall then be able to assay substances for their inhibitory value. This would greatly facilitate the study of gastric cancers." (100, p.406)

The rats were obtained from the University Clinic of the Royal Victoria Hospital. The exact geneology of this strain remains unknown, but it is believed that these Hooded, or piebald, rats are the off-spring of Wistar rats and Norway wild rats (195). The colony has been highly inbred from continuous brother and sister matings since 1933. A control group of 30 male rats and 30 female rats was kept for one year by Dr. Leon Heller in the Department of Experimental Surgery to note the occurrence of spontaneous tumors. No gastric tumors were found. Although the principal aging disease in this strain of rats is pulmonary infection, the young animals were all in good health at the time of operation.

Since Lewis and King (119) found that tumors developed sooner in male rats than in females regardless

of the strain, it was decided to use male rats for this work. It is believed that this difference is associated with the more rapid growth of the males. Females of the Royal Victoria Hospital colony weighing an average of 29.3 gms. at 22 days of age, weighed only 132.3 gms. at 53 days after birth, whereas males of the same litter that weighed 32.6gms. at 22 days of age, weighed 183.0 gms. at 53 days after birth.

Stainless steel cages of the hanging type with half-inch wire mesh bottoms, which allow the feces to pass through to sawdust pans below them, were used for these rats. The cages have been cleaned and sterilized every 4 weeks. Water and food, Purina Fox Meal, were provided ad libitum. The room was ventilated with a window fan-ventilator. In February, 1951, a thermostatic heat control regulator was installed to keep the temperature at 80°F., because Wallace et al. (228) have proved that methylcholanthrene-induced tumors grow more rapidly in a warm environment.

The male rats used for the operations were chosen on the basis of weight rather than age, because it was found that the stomachs of rats weighing less than 150 gms. before operation were too small to handle easily, while rats weighing more than 200 gms. before operation had too much fat is the mesentery so that the right gastro-epiploic artery could not be seen clearly at operation. These rats were all between 2 to 3 months of age at the time of operation. On the day before operation, the food was removed from the cages, but water was allowed ad libitum up to the time of operation.

IV GASTRIC ANATOMY OF THE RAT

In order to explain the operative procedure done on these rats, it is necessary to describe in some detail the gastric anatomy of the rat.

The rat's stomach is in the same position in the abdominal cavity as the human stomach. There are, however, several marked differences (53). For example, a small lobe of the liver fits snugly around the lower end of the esophagus like a collar, and, bulging up to the left, is

the thin walled, grayish-white, translucent forestomach, or ruman (Fig. 1). The forestomach, which is approximately two-fifths of the total area of the stomach, ends abruptly at the limiting ridge (184). The body, or glandular portion, of the stomach is thick walled and similar in color to the human stomach. The greater and lesser curvatures end at a definite pylorus. The duodenum is not "C" shaped and retroperitoneal as in the human, but has a well formed mesentery.

The arteries supplying the rat's stomach are similar in arrangement to those supplying the human stomach with one important exception; there is no left gastro-epiploic artery (Fig. 2 & 3). The right gastro-epiploic artery , coming off from the gastroduodenal artery, runs in the mesentery along the greater curvature and supplies the glandular portion of the stomach along the greater curvature. It stops abruptly at the limiting ridge. Greene mentions, in her excellent monograph on "The Anatomy of the Rat "(84), that she encountered great difficulty in tracing the course of all the arteries from the coeliac axis unless

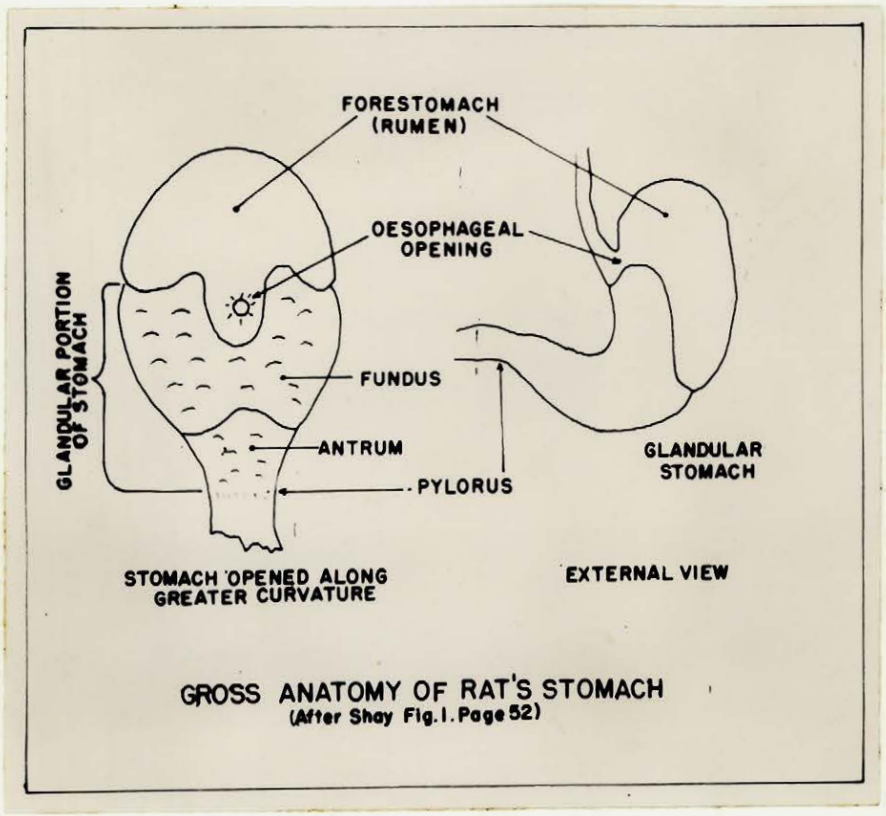

Fig. 1. Gross anatomy of the rat's stomach.

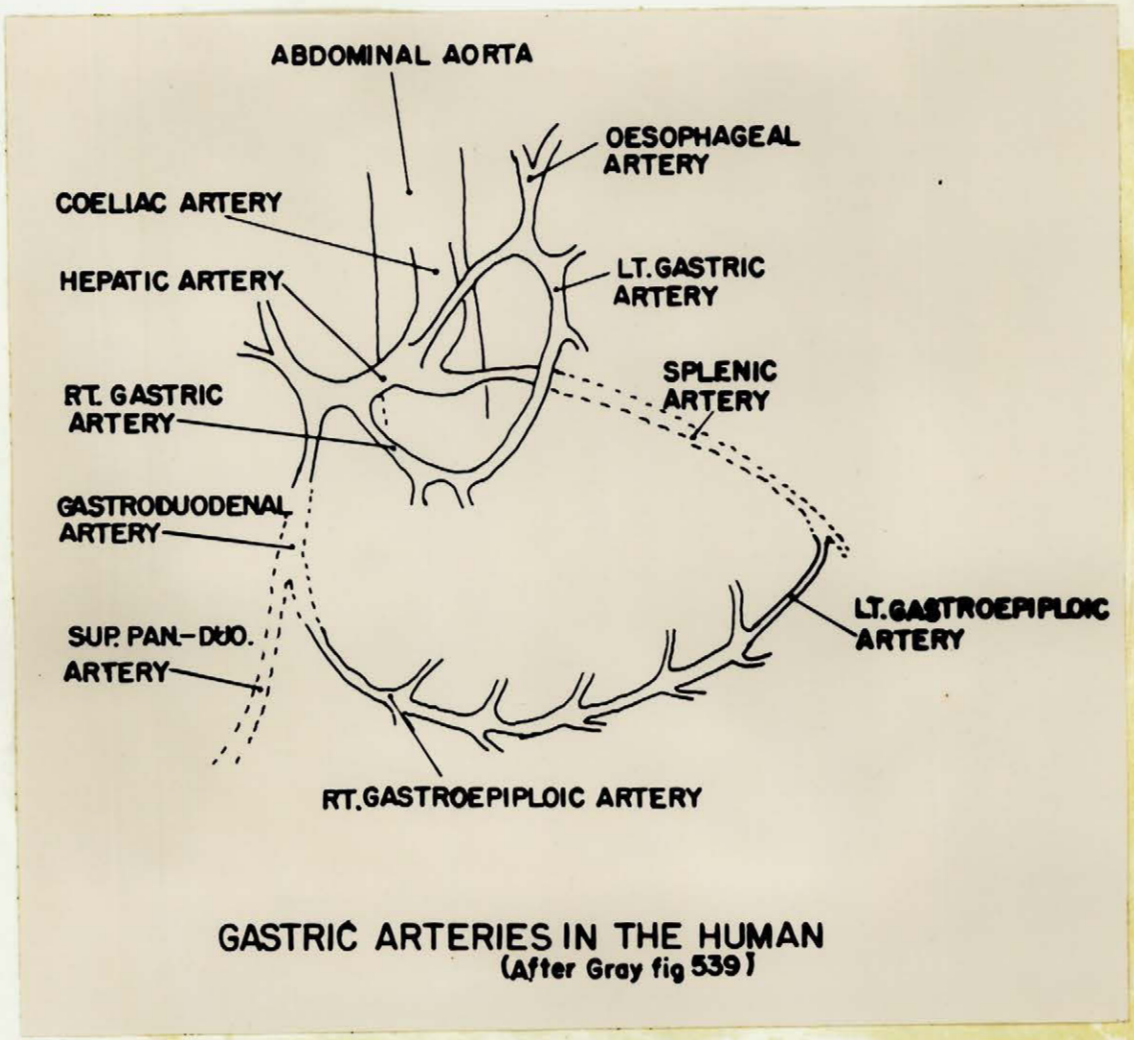

Fig. 2. Gastric arteries in the human.

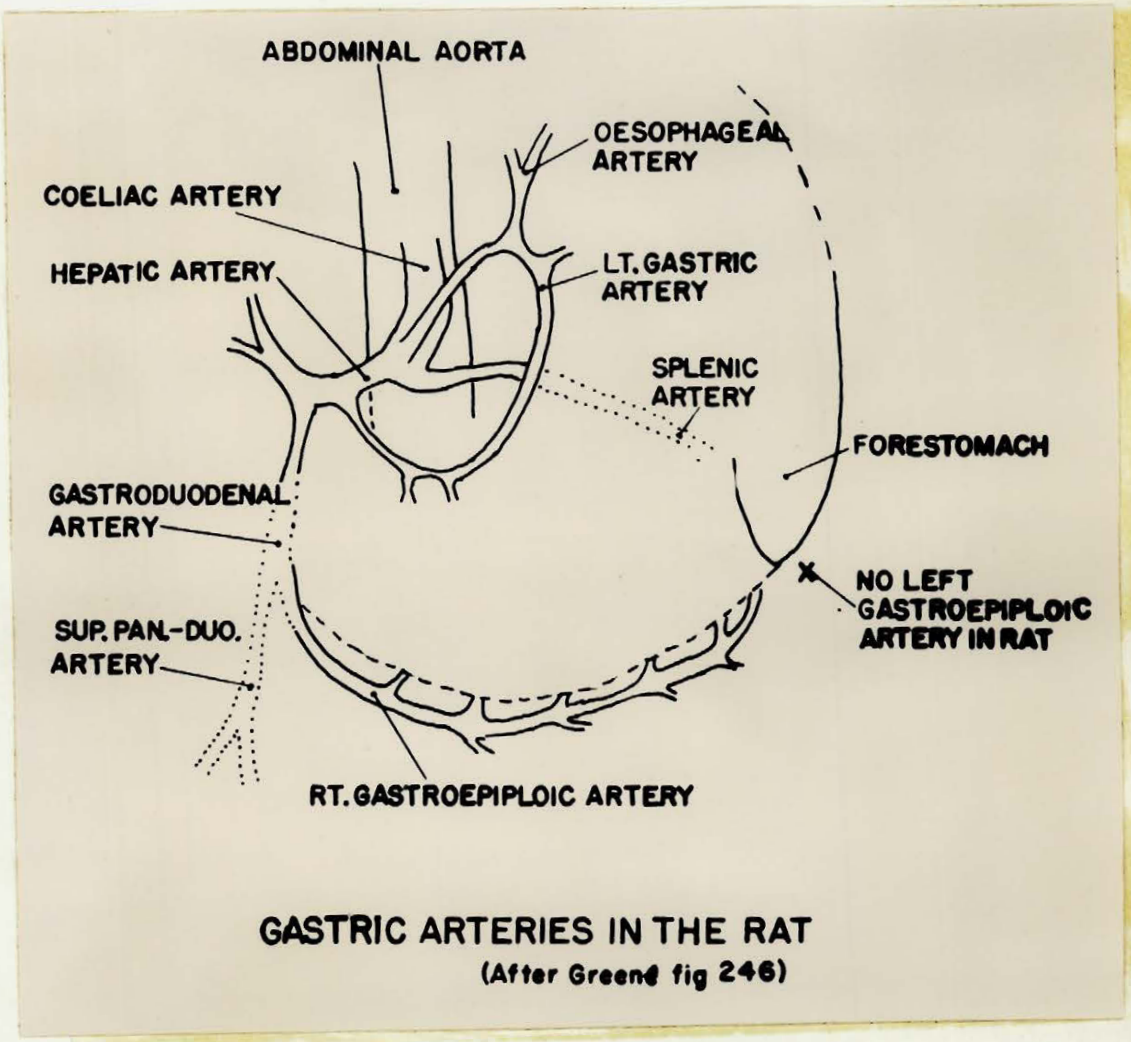

Fig. 3. Gastric arteries in the rat.

the specimen was especially injected for that purpose. From her drawings and our dissections, the arteries to the stomach of the rat appear as in Fig. 3 in comparison with the arteries to the human stomach as shown in Fig. 2.

When the stomach is opened along the greater curvature and washed out, the forestomach, or rumen, appears smooth and glistening. It ends abruptly in a raised white-edged ridge, which dips down onto the lesser curvature to encircle the opening of the esophagus (Fig. I). The glandular stomach appears deep pink, ridged, and velvety. It is divided into a corpus and antrum. In rats under 2 to 3 months of age, this division is not so distinct as in older rats, where the antral mucosa is smoother and, where small, whitish nodules of lymphoid tissue can be seen near the pylorus (18).

V OPERATING TECHNIC

The instruments, sutures, sponges, and drapes are all sterilized for 15 minutes in an autoclave at 15 lbs. pressure just before the operations are started, but are not sterilized again during a session of 3 or 4 opera-

tions. No sterile gowns, masks, or gloves are used, since a brief hand scrub has been found sufficient to prevent peritonitis or stitch abscesses (Fig. 4 & 5).

The rat is anesthetized lightly with ether in a glass jar, removed, and tied to the operating board. The anesthesia is continued intermittently by using a small 50 cc. beaker as an ether cone. A layer of cotton placed in the bottom of the beaker is moistened with ether. The hair is shaved off the left upper quadrant, tincture of merthiolate applied, and a small sterile drape sheet is placed over the rat with the edges of the sheet tucked in under the board. A left rectus muscle-splitting incision starting just below the costal margin is extended downward for 3 cm. The stomach is delivered into the incision with a smooth forceps, and the mesentery on the right side of the greater curvature is held up to the light (Fig. 6 & 7). The arcades of the right gastro-epiploic artery can then be seen well so that a clear space can be perforated with a fine pointed forceps (Fig. 8 & 9). The tip of one blade of a curved Halstead hemostat is then inserted through

Fig. 4. View of the operating table.

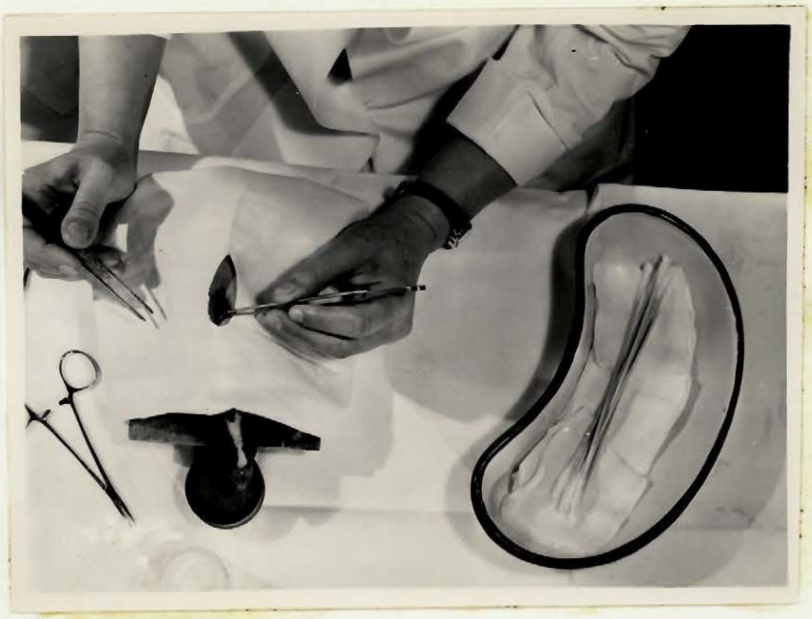

Fig. 5. Beginning of the operation.

Fig. 6. Gross specimen of the stomach with mesentery.

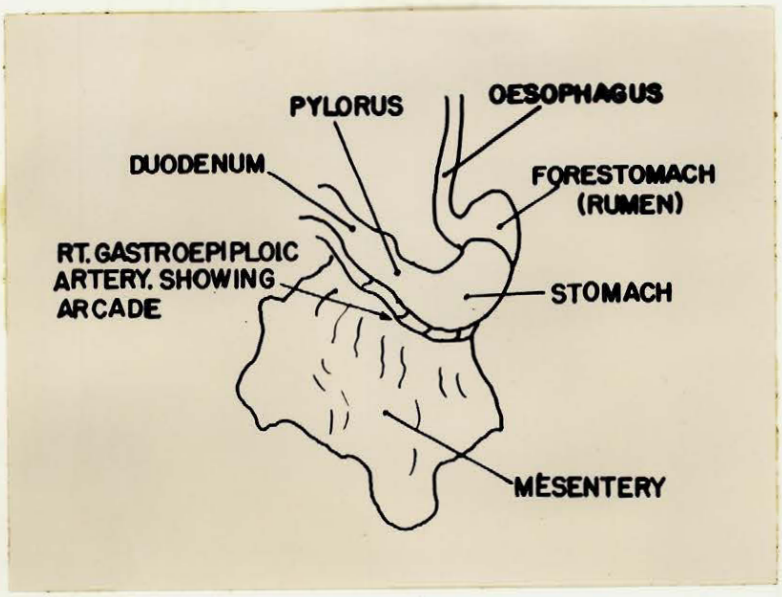

Fig. 7. Diagram of the stomach with mesentery.

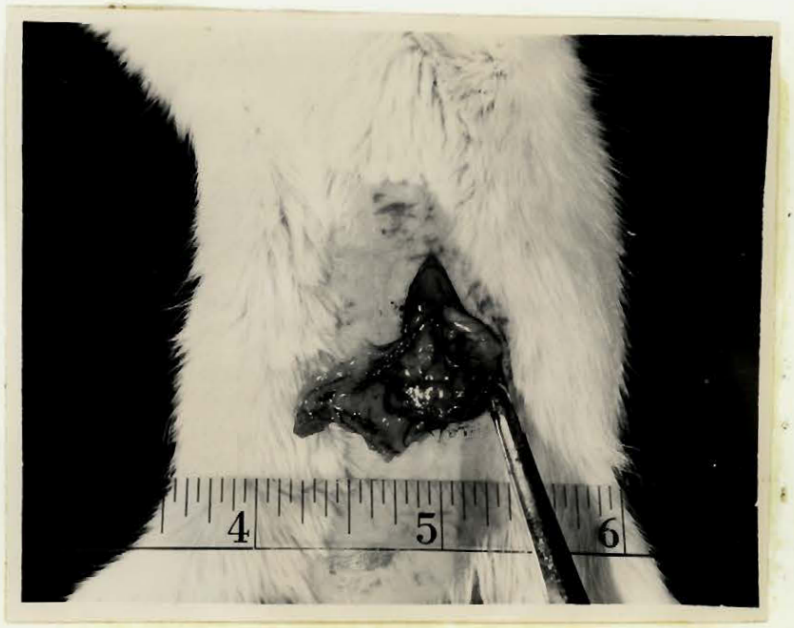

Fig. 8. Stomach delivered into the incision.

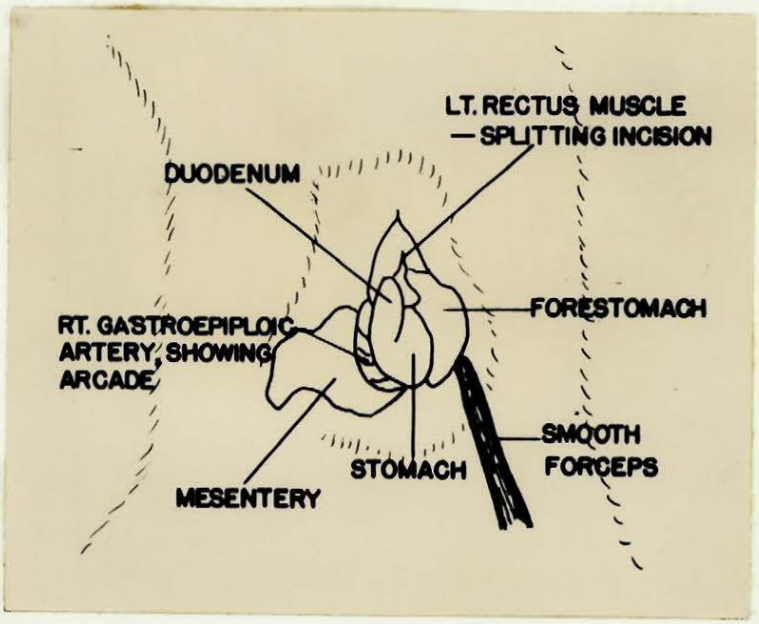

Fig. 9. Diagram of stomach delivered into the incision.

this hole, and the blades are closed tightly, thereby crushing together a very narrow strip of the anterior and posterior walls of the stomach. This procedure leaves the blood supply via the right gastro-epiploic artery intact to the greater curvature, which will constitute the flap, and, by crushing the tissue, provides hemostasis on the cut edges. The blades of the curved Halstead hemostat used in this operation were filed down to 1 mm. in width. When placing the hemostat before the final closure, care must be taken to include an adequate segment of glandular stomach without any squamous forestomach, and not to impinge on the pylorus. Four No. 60 black cotton sutures are then placed in the greater curvature close to the hemostat to serve as stay sutures in the four corners of the future flap (Fig. 10 & 11). The ends are left 8 cm. long. A scalpel is used to sever the greater curvature from the stomach while the stay sutures are held taut so that the mesentery carrying the arterial supply is not damaged with the scalpel. Then the detached portion, i.e., the greater curvature, is laid to the right of the incision on the abdominal wall

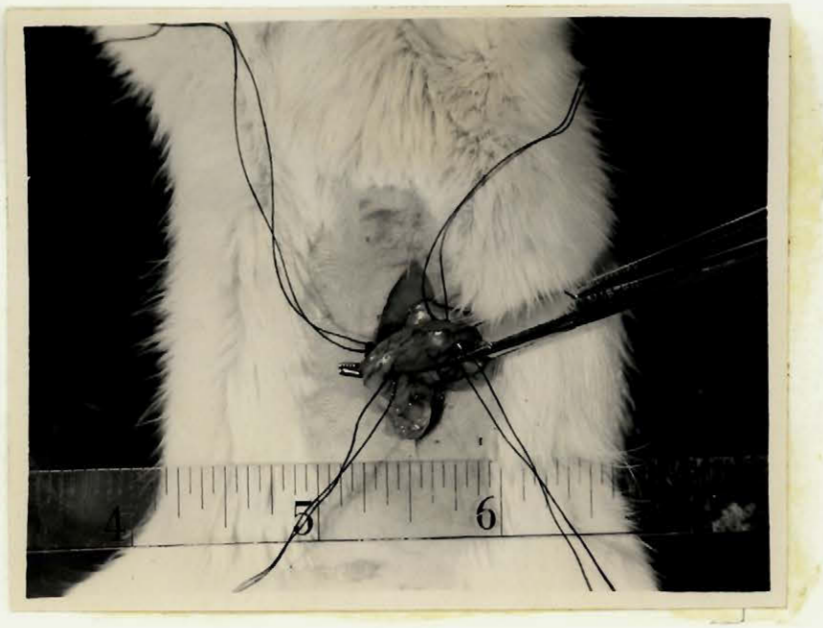

Fig. 10. Greater curvature crushed with Halstead hemostat.

Four stay sutures are in place.

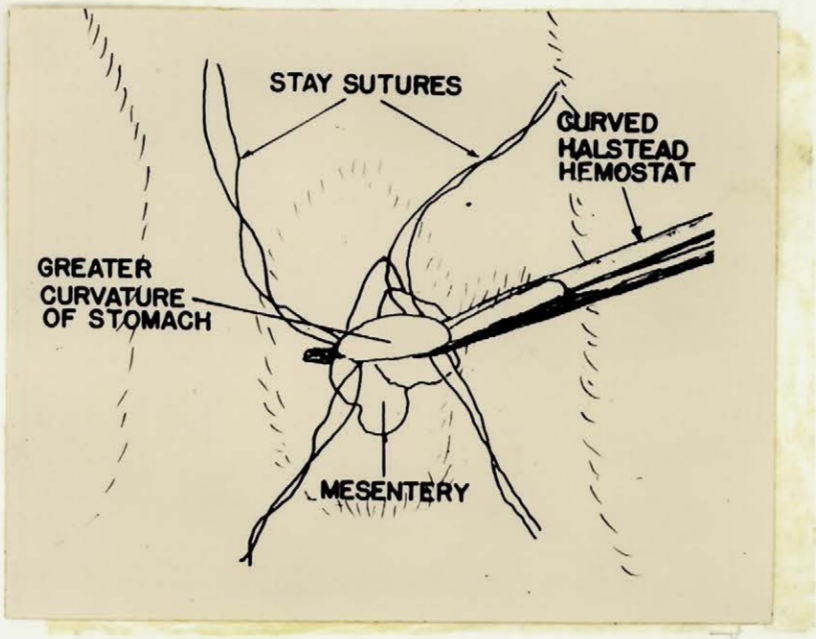

Fig. 11. Diagram to illustrate Fig. 10.

and covered with a sponge moistened in warm saline solution (Fig. 12 & 13). A Parker-Kerr method of closure over the hemostat on the cut edge of the stomach is then done with a five-zero twisted black silk artery suture on a curved atraumatic needle (Fig. 14, 15, 16, & 17). Several interrupted reinforcing sutures of the same material are placed along the line of closure (Fig. 18 & 19), and the stomach is dropped back into the abdominal cavity. The split left rectus muscle is closed with interrupted sutures of No. 60 black cotton above and below the mesentery which comes out of the abdominal cavity and carries the blood supply to the flap (Fig. 20 & 21). After the moist gauze has been removed from the flap, the stay sutures are separated, and the flap is laid in place with the mucosa side out, over the closed rectus incision. The stay sutures at the four corners are then used to anchor the flap to the muscle of the abdominal wall (Fig. 22 & 23). The skin edges are pulled over to fit snugly around the flap and are sutured in place with No. 60 black cotton by catching the muscle wall beneath them (Fig. 24 & 25).

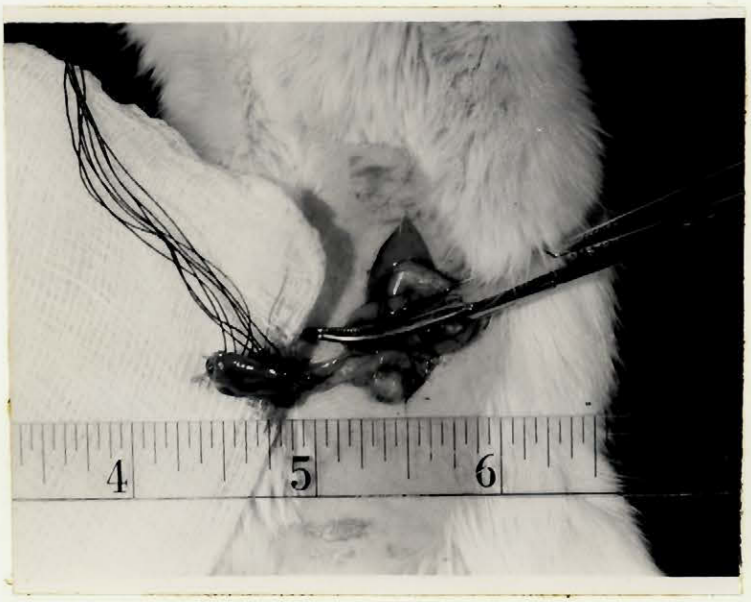

Fig. 12. The flap has been severed from the stomach.

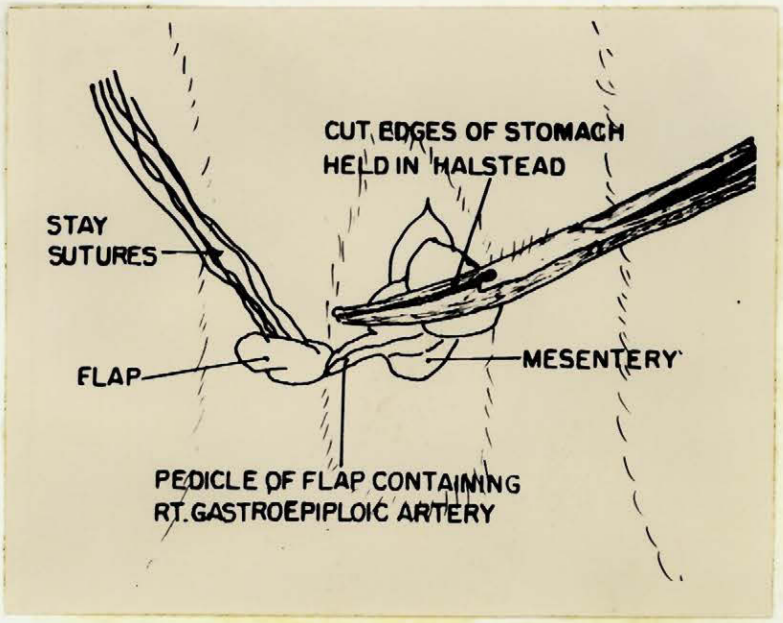

Fig. 13. Diagram to illustrate Fig. 12.

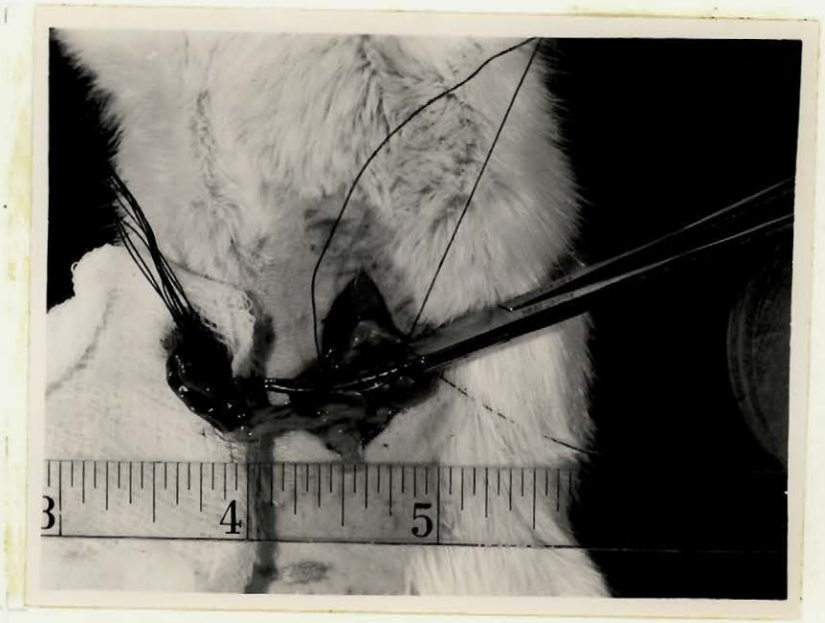

Fig. 14. Insertion of the Parker - Kerr suture.

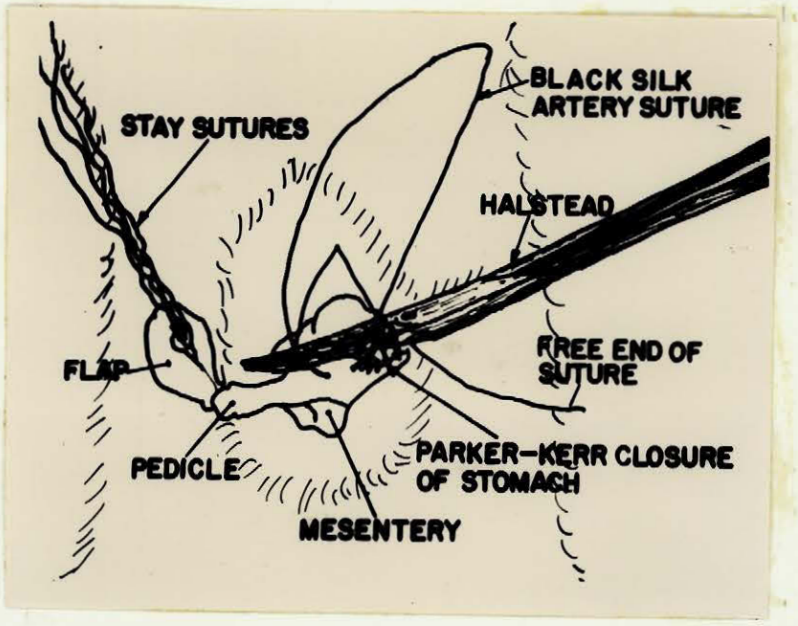

Fig. 15. Diagram to illustrate Fig. 14.

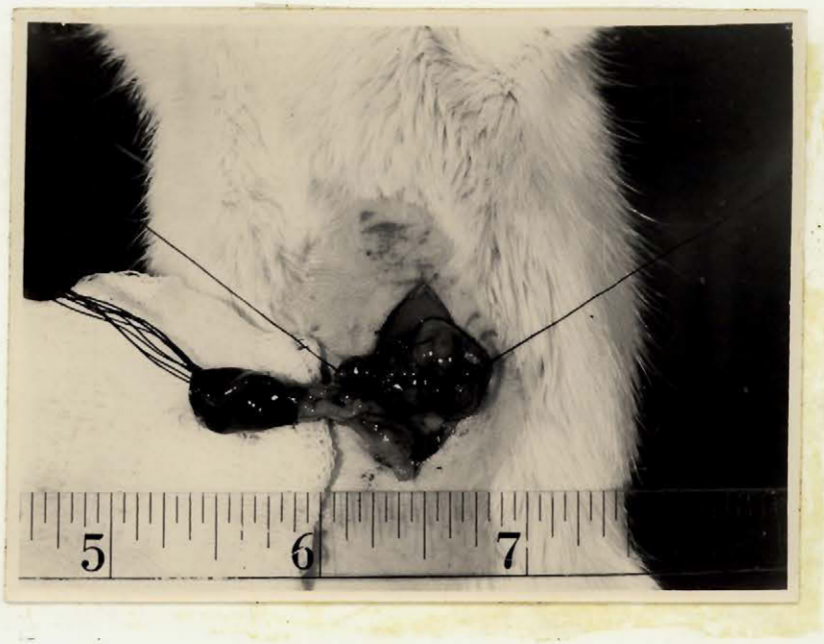

Fig. 16. The stomach edges are inverted by the Parker - Kerr suture.

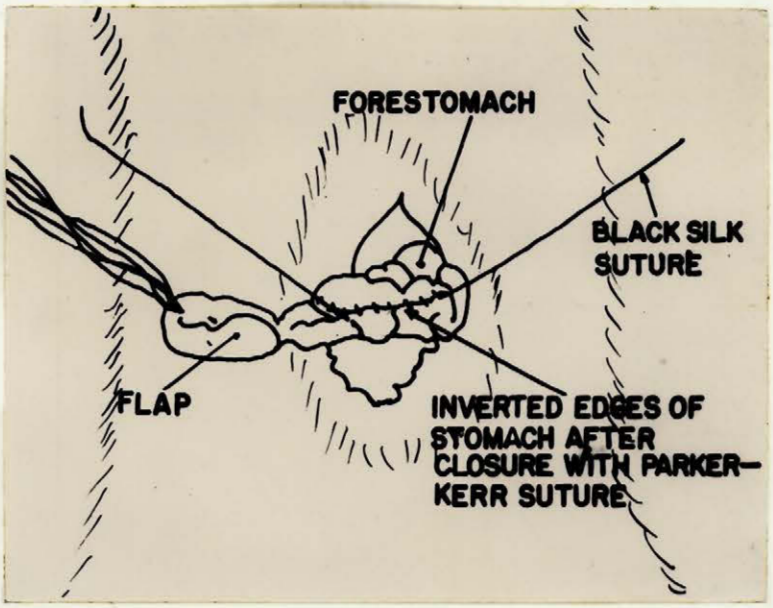

Fig. 17. Diagram to illustrate Fig. 16.

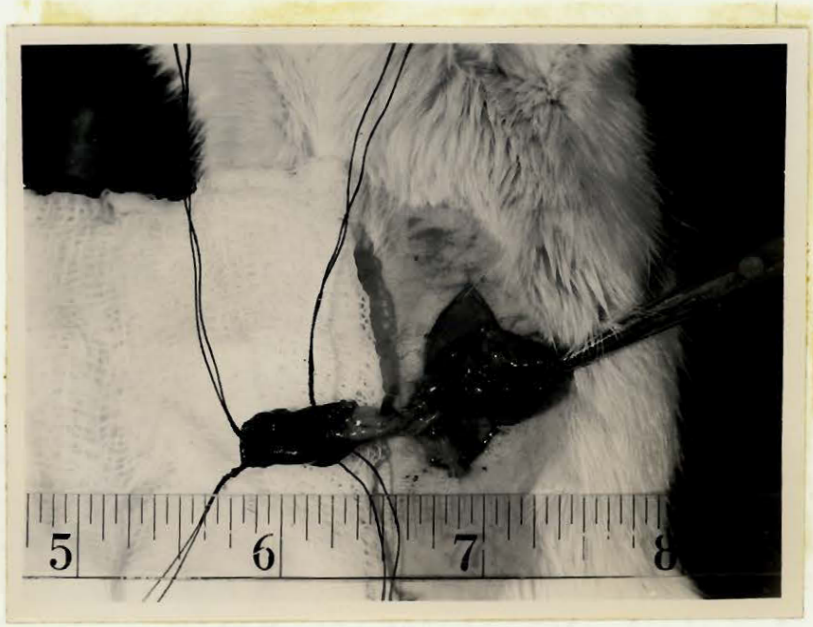

Fig. 18. Reinforcing sutures along the line of closure.

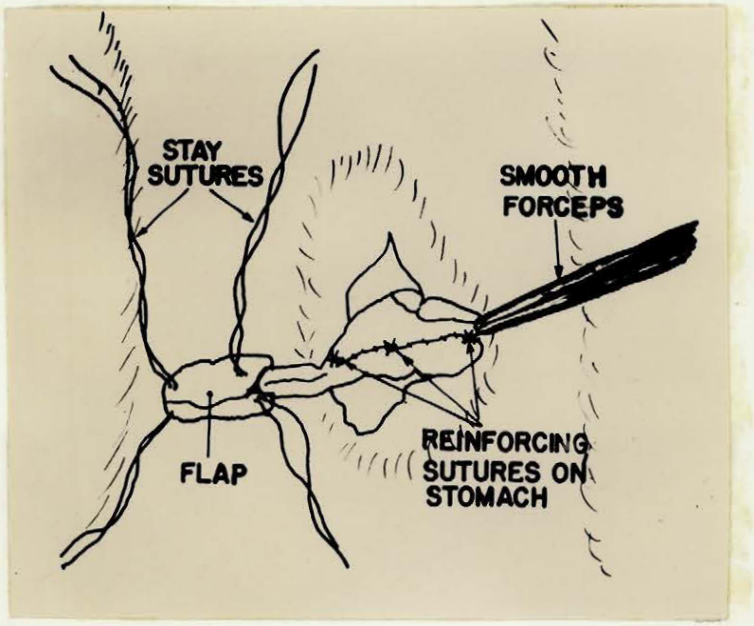

Fig. 19. Diagram to illustrate Fig. 18.

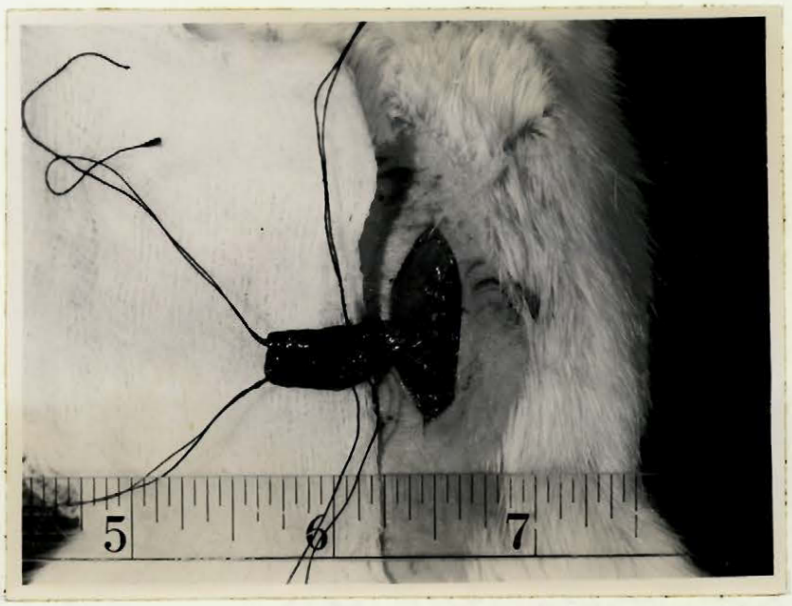

Fig. 20. The split left rectus muscle is closed.

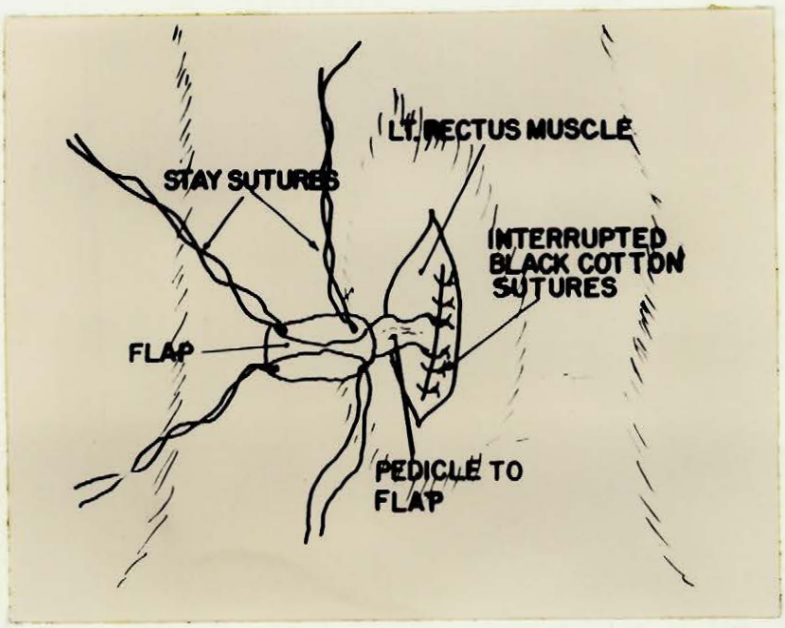

Fig. 21. Diagram to illustrate Fig. 20.

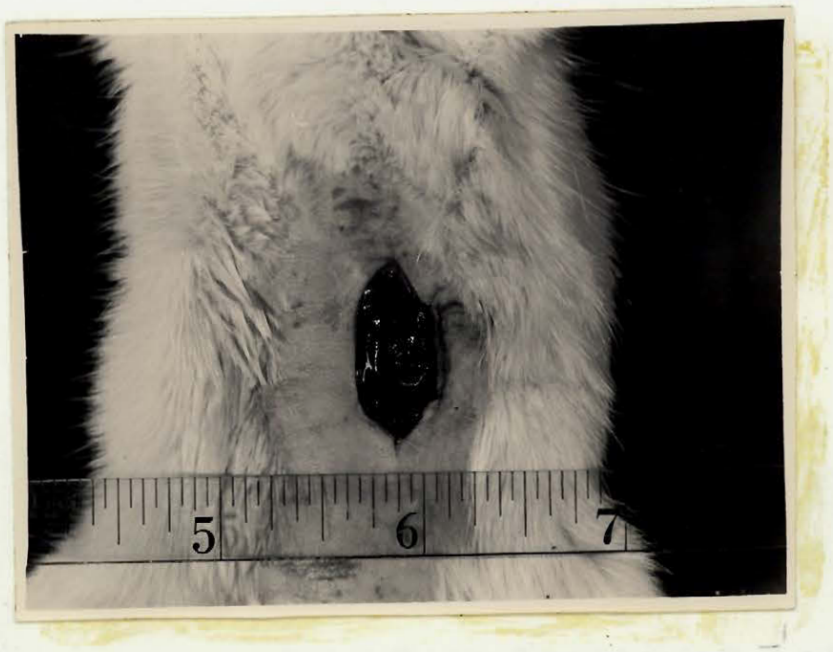

Fig. 22. The flap is anchored to the abdominal wall.

Fig. 23. Diagram to illustrate Fig. 22.

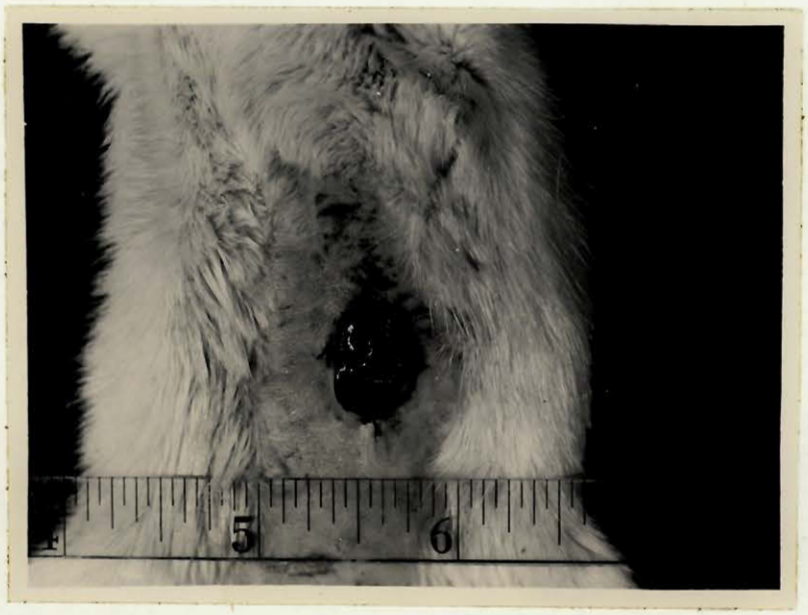

Fig. 24. The skin edges are sutured snugly around the flap.

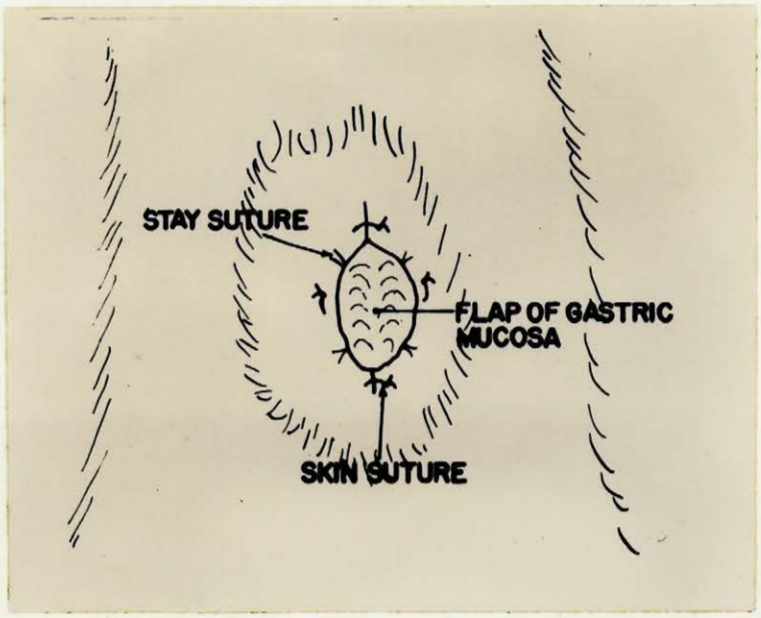

Fig. 25. Diagram to illustrate Fig. 24.

The operating time for this procedure without an assistant is 30 minutes. With a well trained assistant, the time can be cut to 20 minutes.

There is very little blood lost during the operation, and the animals are active and drink water within a few minutes. No food is given until 24 hours after the operation.

Skin sutures are removed on the seventh p. o. day, because stitch abscesses develop if the sutures are left in place. By the tenth p. o. day the flaps are well established, and the painting with carcinogens can be started.

VI UTILIZATION OF THE FLAPS

In order to see if the operative technic just described was a practical one, it was first performed on 20 rats. All the animals survived the operation, and in 17 of the rats the flaps grew well. The flaps of 3 animals dried up slowly over a period of 3 to 4 days. The 17 successful flaps were then used for testing various painting technics and vehicles. When the carcinogens, vehicles, and procedure of application, which will all be discussed later, were

chosen, operations to give the required number of animals with flaps were started on July 17, 1950 and completed by October 14, 1950. During the months of June, July, and August, Dr. Leon Heller, who was then a medical student working on a Cancer Research grant, performed half of the operations and assisted with all of the work.

SUMMARY OF OPERATIONS

Successful flaps	123	67.5%
Deaths	10	5.5%
Discards	49	27.0%
Total number of operations	182	100.0%

Deaths: Of the 10 animals which died in the immediate post-operative period, 7 were found to have pyloric obstruction, and 2 had multiple kidney abscesses at post-mortem examination. One animal died without coming out of the ether anesthesia.

Discards: Forty-nine animals were discarded from the experiment because the flaps of gastric mucosa disappeared. This atrophy of the flaps occurred during the first 10 days after operation and was due to damage of the right gastro-epiploic artery during the operation. In these animals, the flap either shrivelled up in 24 hours or became infected and sloughed off. The skin defect closed, and the hair grew again covering the incision. These animals were all killed with ether and examined, but no abnormalities were found.

It was of interest to find in later work that flaps which had been growing for several months did not depend entirely on the right gastro-epiploic artery for blood supply. When this artery was completely severed, the flap blanched and remained pale for several hours, but blood vessels which had grown in from the surrounding tissue were able to supply sufficient blood to keep the flap in good condition.

Successful flaps: There were 123 successful flaps, and of these, 120 were used for the experiment. The skin healed well and grew up snugly to the edge of the flap of gastric mucosa without growing over it. By the 10th post-

operative day, the skin edges were completely healed, and the animals were ready for use in the experiment.

VII CARCINOGENS

A. METHYLCHOLANTHRENE

The cancer-producing properties of certain substances were first noted by Percivall Pott (162) in 1775, who drew attention to the high incidence of cancer of the scrotum in chimney sweeps. He suggested that this was due to some special quality of the soot to which they were exposed. It was not until 1914, however, that the first experimental cancer was produced by Yamagiwa and Ichikawa (242) in the ears of rabbits painted with crude tar. The production of experimental cancer with crude substances was never consistent, but from the test discovered by the physicist, Mayneord (130, 37), in 1927, that carcinogenic tars produced similar bands in the fluorescence spectrum, Hieger (89), utilizing these bands as guides, was able to concentrate the active material in coal tar pitch. With the important discovery by Kennaway, Hieger, and their associates (104), in 1930, that certain pure hydrocarbons

possess the specific property of producing cancer in animals, new impetus was given to the work on experimental carcinogenesis. During this early work on the synthesis of polycyclic hydrocarbons, Cook (36), in 1933, predicted the formation and even the carcinogenic potency of a compound which he believed could be derived from deoxycholic acid. This, he found to be methylcholanthrene.

From among the many carcinogenic hydrocarbons which have been synthesized and tested in the past 20 years, the choice of methylcholanthrene as the principal hydrocarbon for this work was not fortuitous. Methylcholanthrene has been produced from cholestrol (9) which is a normal constituent of food (115, 105, 106, 107) and can, therefore, be considered among the extrinsic factors in the cause of gastric cancer. It has also been produced from bile acids (38, 39, 40, 71) and has, for this reason, been considered one of the intrinsic factors in the problem of gastric cancer. Moreover, the changes, by which methylcholanthrene is obtained from deoxycholic acid, are all reactions which are known to

occur normally in the animal body (14) (Fig. 26).

Methylcholanthrene is one of the most potent carcinogens (226, 17). Badger (9) has stated that: "The cholanthrenes, and related compounds, are most conveniently considered as substituted benzanthracenes, although the sterol numbering often used tends to confuse the relationship. Cholanthrene, 20-methylcholanthrene, 22-methylcholanthrene and 23-methylcholanthrene have been prepared and tested. They may be considered as benzanthracenes substituted in position 10-, 5-, and one other position. All were found to be very active, but there is little doubt that 20-methylcholanthrene is the most active, then cholanthrene, then 22-methylcholanthrene, and then least active, 23-methylcholanthrene." (9, p. 317) Except in papers which discuss the chemical composition or the synthesis of polycyclic hydrocarbons, 20-methylcholanthrene by usage is designated as methylcholanthrene.

Following the discovery of methylcholanthrene by Cook and Haslewood (38), and, independently by Wieland and Dane (236), in 1933, various processes were used for its

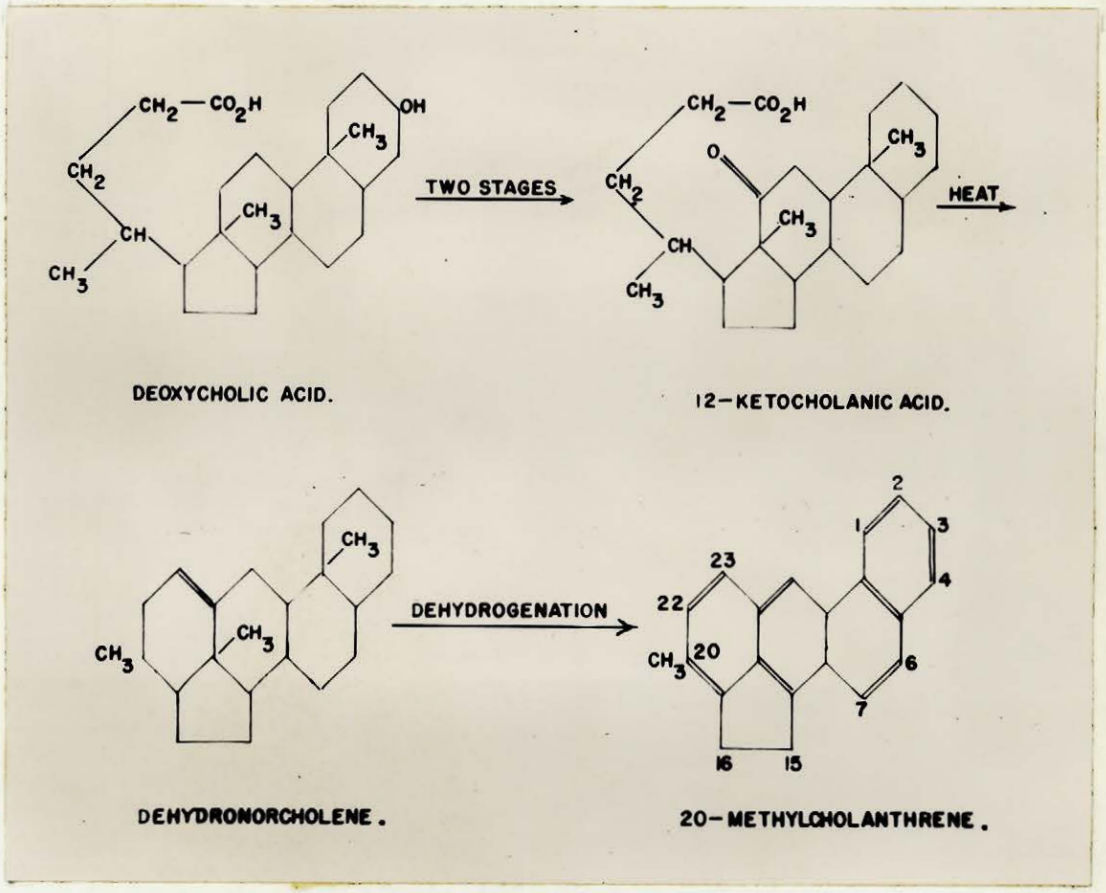

Fig. 26. Steps in the formation of 20-methylcholanthrene from deoxycholic acid.

synthesis. These have been reviewed in detail by Fieser (70). The improved synthesis developed by Fieser and Seligman (72, 73, 74) is used commercially. Pure methylcholanthrene can be prepared rapidly on a large scale in 6 steps from p-chlorotoluene with an over-all yield of 20 per cent. It is in the form of light yellow needles, having a melting point of 179.5 - 180.0° C. corr. Methylcholanthrene has been used extensively in experimental carcinogenesis, although the mode of its action in causing cancer is still unknown (60, 137). Cook and Kennaway (41) reviewed all the experimental work published up to 1941 using methylcholanthrene as a carcinogen. This covered only the few years since its discovery in 1933, and by that time there were over a thousand publications. Since then, methylcholanthrene has been widely used as a carcinogen in tissue culture work (60), mold growth experiments (10), and genetics (222), in addition to the vast work on experimental animals. The percutaneous application of methylcholanthrene has caused squamous cell carcinoma

of the skin (55, 136), adenocarcinoma of the breast (62, 16), and leukemia (235). When methylcholanthrene was injected subcutaneously, or into solid organs such as the thyroid, kidney, liver, spleen, and testis (63), sarcomas resulted. After intravenous injection, methylcholanthrene has caused adenomas of the lung (191). Intraperitoneal injections of methylcholanthrene adsorbed on activated carbon produced reticulum cell sarcoma in mesenteric lymph nodes (133). Methylcholanthrene-cholesterol pellets inserted into the brain caused gliomas (175) and implanted into the medullary cavity of the tibia or femur caused fibrosarcomas of the bone to develop(27). Oral administration produced squamous cell carcinoma of the esophagus, adenocarcinoma of the small intestine (124, 125), adenocarcinoma of the breast, and leukemias (185, 186). In spite of this wide range of carcinogenic activity, adenocarcinoma of the glandular stomach has been very difficult to obtain.

In mice, adenocarcinoma of the glandular stomach was first obtained by Stewart and Lorenz in 1942 (213) by injecting a dispersion of methylcholanthrene in horse serum

into the submucosa of the glandular stomach. They obtained 11 gastric adenocarcinomas in 293 mice. Stewart, Hare, Lorenz, and Bennett (216) repeated this experiment in 1949 and obtained 8 adenocarcinomas in 250 mice so injected. Stewart and Lorenz (214) also obtained several adenocarcinomas in the glandular stomachs in mice in a later experiment by using Andervont's technic (4) of implanting methylcholanthrene impregnated threads into the submucosa. These gastric adenocarcinomas in mice developed in 2 to 11 months after treatment with methylcholanthrene.

In rats, adenocarcinoma of the glandular stomach has been much more difficult to produce. In 1948, Howes and de Oliveira (98) stated they had obtained adenocarcinomas in rats by implanting methylcholanthrene impregnated threads between the gastric mucosa and serosa. They used 150 rats in their experiment, but unfortunately neglected to state how many gastric adenocarcinomas were obtained, or how long a time passed before the neoplasms developed. Dr. Antonio Cantero, in a personal communication (35) has said that he has been able to repeat Howes' work in rats. This report has

not been published yet. Dr. Harold L. Stewart (212) has treated 265 rats by injecting methylcholanthrene dispersions in horse serum into the fundic and pyloric regions of each stomach (217). He obtained 4 adenocarcinomas in the pyloric area with metastases. These neoplasms appeared in 12 to 14 months after injection. This work will soon be published in the "Journal of the National Cancer Institute."

Very little is known of the method of absorption and excretion of methylcholanthrene in the animal body. In 1947, Larionow (116), using spectrographic methods, stated that methylcholanthrene is absorbed from the forestomach and glandular stomach in rats and mice into the blood stream and can later be found in the liver. Setälä and Ekwall (176, 177), in 1950, used the same water- and lipoid-soluble polyethylene glycols as were used in this work, and they were able to follow the penetration of benzpyrene, a carcinogen similar to methylcholanthrene, into the glandular stomach of mice by the fluorescent microscope technic. The gland cells appeared to take the carcinogen into their cyto-

plasm. From there, it appeared to go into the lymph channels. These results are only a preliminary report, and the authors state that later work will discuss the excretion of benzpyrene and other carcinogenic hydrocarbons.

B. 2-ACETYLAMINOFLUORENE

A second carcinogen, 2-acetylaminofluorene, which produces a great variety of distant tumors (237, 238), was also used in one series of flap animals as an exploratory investigation. In the Department of Experimental Surgery, Dr. Stanley C. Skoryna has completed a long investigation on the effect of feeding 2-acetylaminofluorene to the Royal Victoria Hospital Hooded rats. He has produced numerous hepatomas with this carcinogen and many other distant tumors. The extensive literature on 2-acetylaminofluorene was reviewed by him in his Master of Science thesis written in 1950 on "Effects of 2-Acetylaminofluorene on the Liver in Rats." Feeding experiments were also carried out in the Department by Dr. Leon Heller using 2-acetylaminofluorene which produced hepatomas.

Morris et al. (143), in 1950, reported that when the skin of rats was painted with a 4 per cent solution of 2-acetylaminofluorene in acetone, the painted animals survived much longer than the rats ingesting this compound. In the rats painted with the 2-acetylaminofluorene, 78 per cent developed tumors, while only 60 per cent of the rats ingesting this carcinogen developed tumors. No adenocarcinomas of the glandular stomach were produced in this experiment, although Wilson, De Eds, and Cox (238) reported finding one gastric adenocarcinoma after feeding 2-acetylaminofluorene to many hundreds of rats in their experiments. From their work, they believed that malignant tumors were usually located in areas where irregular hyperplasia of the cells was originally present.

It was of interest, therefore, to paint the hyperplastic flaps of gastric mucosa with 2-acetylaminofluorene for two reasons; first, to see if sufficient absorption of 2-acetylaminofluorene could be obtained through the flaps to produce distant tumors; and second, to see if the irregular

hyperplasia already present in the flaps could be stimulated to malignant change.

VIII VEHICLE

The problem of choosing a suitable solvent for the carcinogens used in this work was a difficult one. The usual solvents such as benzene, acetone, or ether, when painted on the flaps of gastric mucosa caused the rats to run frantically around the cage, then sit up, and lick the flaps and surrounding skin vigorously until the irritation had subsided. The use of one of such irritating solvents as a vehicle for the carcinogen would obviously defeat any attempt to apply the carcinogen locally.

Although these solvents are employed in many skin-painting experiments as vehicles for the carcinogen (20, 42), an area between the scapulae is painted so that the animals cannot remove the carcinogen by licking the irritated region.

Bland solvents, such as lard, mouse-fat, and lanolin, which would not irritate the flaps, have also been

used in experimental skin carcinogenesis (47). Lanolin, however, destroys the carcinogenic activity of methylcholanthrene (193, 194), whereas mouse-fat and lard both exert a definite deterrent effect on carcinogenesis (51, 58). Fieser (70), in 1938, Rusch (166), in 1944, and Peacock et al. (158), in 1949, reviewed in detail all the solvents used as vehicles for carcinogenic hydrocarbons up to that time.

The use of suspensions of the carcinogenic hydrocarbons in water and glycerol (63), as well as emulsions in olive oil and mineral oil (215, 123), and dispersions in cholesterol (190), were also investigated. However, the technical difficulty of keeping the amounts of the hydrocarbon uniform for each application prohibited their use for painting. An additional contra-indication was the low absorption of any particulate substance applied in such a manner.

Many other workers in this field have had the same difficulty in obtaining a bland, stable vehicle. In

fact, Ekwall and Setälä (61), in 1949, after reviewing thoroughly the extensive literature on solvents, described a new method whereby a clear, absolutely homogenous, and stable solution can be obtained. They used the following three carcinogenic hydrocarbons: 20-methylcholanthrene, 9-10-dimethyl-1, 2-benzanthracene, and 1, 2, 5, 6-dibenz-anthracene. Many colloid-forming substances were investigated, and they concluded that sodium oleate, sodium cholate, and Triton NE, (the colloid forming substance is an alkyl, aryl polyether alcohol), gave the best results. They state that: ". . . in some cases the solubilities of the hydrocarbons increase in the same proportion as the concentration of the colloid (oleate, Triton NE) and in other cases as the quadrate of the colloid concentration (cholate). There is some parallelism between the solubilities of the three hydrocarbons investigated in a given colloid and their carcinogenic action." (61, p. 738)

This method seems to hold great promise, but as yet, scant work has been published on the toxicity to laboratory animals (176), and until that has been thoroughly

investigated, it was believed unwise for use in this work.

In addition to the blandness and stability of the vehicle, another characteristic was of importance. The vehicle had to be readily absorbed by the tissue to be studied (50). Barrett (12), in 1949, pointed out that too frequently in experimental carcinogenesis, the gastric mucosa, which is noteworthy for being hydrophilic, is exposed to lipophilic carcinogens, and suggested that a search should be made for "carcinogenic substances that are more adaptable to this type of (hydrophilic) tissue." (12, p. 561) Strait, Hrenoff, and De Ome (220), in 1948, had already encountered a similar problem with lipoid solvents, and concluded that the influence of the solvent as a vehicle affects the availability of the carcinogen. They suggested that some method of using the serum of the experimental animal as a vehicle might overcome the difficulty of using a lipoid solvent.

For this experiment, however, in which painting had to be carried out three times each week over a period of a year or more, serum as a vehicle was obviously impractical.

In summary, then, the problem was to find a vehicle with the following characteristics:

1. It had to be a solvent for the carcinogenic hydrocarbons, but at the same time not deter carcinogenesis.
2. It had to be non-irritating so that the rats would not remove it immediately by licking.
3. It had to be stable in order to deliver a uniform amount of the carcinogen at each painting.
4. It had to be hydrophilic to be absorbed readily by the gastric mucosa.
5. It had to be tested thoroughly on experimental animals to determine its toxicity before it could be used in this work.

A vehicle satisfying all these demands was finally found in polyethylene 300.

Historical:

Polyethylene glycols were first prepared by Lourenco (126) in 1859. No important work was done on them,

however, until Staudinger (207), in 1932, proposed that very high polymeric substances of the polyethylene oxide and cellulose types exist in solution as long macromolecules. Spurred by the industrial importance of the preparation of synthetic fibers and plastics from these substances in the early 1930s, Hibbert and his associates at McGill (78, 79, 80, 127) made an extensive study of the synthesis of the higher polyethylene glycols.

One aspect of the industrial use of these polyethylene glycols was developed by Carbide and Carbon Chemicals Corporation for emulsifying agents and ointment bases. Before pharmaceutical preparations using polyethylene glycols could be placed on the market, extensive toxicity studies had to be carried out. These were done at the Mellon Institute of Industrial Research, Pittsburgh, Pa., over the past 10 years (200, 201, 179, 180).

Physical and Chemical Properties:

The polyethylene glycols comprise a series of polymers with the general formula:

$$HOCH_2(OCH_2CH_2)_n OH$$

In this formula n is one less than the number of ethylene oxide units employed in building the molecule. Diethylene glycol is the lowest member of the series represented by this formula (203). The polyethylene glycols with average molecular weights ranging from 200 to 700 are liquids. Those above 1000 in molecular weight are wax-like solids, known under the trade name of "Carbowax" (Carbide and Carbon Chemicals Corporation).

The lower polyethylene glycols are actually mixtures of lower glycols. Each polyethylene glycol complex has been analyzed by ultra-filtration through various types of cellophane membranes, x-ray diffraction patterns, and selective precipitation by barium silicotungstate (181).

The following table, taken from Shaffer, Critchfield, and Nair's paper (181, p. 348), shows the theoretical percentage of lower glycols in PEG 300, the accepted abbreviation for polyethylene glycol 300.

Theoretical Percentage of Lower Glycols

in some Polyglycols

Per Cent by Weight in PEG 300

Ethylene glycol	$HO\ (CH_2CH_2O)\ H$	0.093
Diethylene glycol	$HO\ (CH_2\ CH_2\ O)_2\ H$	0.86
Triethylene glycol	$HO\ (CH_2CH_2\ O)_3\ H$	3.29
Tetraethylene glycol	$HO\ (CH_2CH_2O)_4\ H$	7.67
Pentaethylene glycol	$HO\ (CH_2CH_2\ O)_5\ H$	12.70
Hexaethylene glycol	$HO\ (CH_2CH_2O)_6\ H$	16.26

PEG 300 is a viscous, straw-colored liquid, completely soluble in water and in many organic solvents such as alephatic ketones, glycol esters, and polycyclic aromatic hydrocarbons, a number of which possess carcinogenic properties. PEG 300 has a pH of 7.23. These glycols do not hydrolyze or deteriorate on standing and will not support mold growth.

Toxicity:

In reviewing all the work done during the past 10 years by the various investigators at the Mellon Institute

of Industrial Research on the toxicity of the polyethylene glycols, material which is now completely out of date had to be abstracted. Most of these references, however, have been omitted from this discussion because the latest investigations published in June of 1950 by Smyth, Carpenter, and Weil (203), and Shaffer, Critchfield, and Nair (181) correct the earlier erroneous suppositions (202). The experimental work quoted below has been taken from this paper.

Due to the nature of this experiment, three modes of absorption of the vehicle, oral, percutaneous, and intravenous, were of importance.

1. Oral absorption. The rats were never seen in the process of licking the gastric flaps immediately after the painting with PEG 300 was done, but in grooming, it would be natural for them to lick this area as well as any other skin areas they could reach with their tongues. For this reason the chronic oral toxicity of PEG 300 was of interest. The greatest amount of PEG 300 applied to one rat

in a single painting was calculated as 0.06 cc.(1 minim). If all of this was ingested, a rat, weighing an average of 190 gms. at the time of the first painting would be getting 0.07 gm., or 0.36 gm. per Kg. per day. In the 1948 experiments reported by Smyth, Carpenter, and Weil (203), 7.2 gms. of PEG 300 per Kg. per day were fed to rats for 90 day periods without causing toxicity or any effect on the kidneys or liver on microscopic examination. An enormous quantity, i.e., four times this amount, or 28.8 gms. per Kg. per day, was required to damage the liver and kidneys sufficiently to cause the death of all the animals ingesting this amount over a seven day period. For this reason, it was felt that the negligible amounts our rats could possibly ingest were well within safe limits of oral absorption.

2. Percutaneous absorption. In painting the flaps, the adjacent skin could occasionally be touched by the brush containing PEG 300, and in grooming, the rats could spread PEG 300 to the skin. No skin penetration toxicity tests with PEG 300 have been done on rats, but in the subacute

inunction applications of PEG 300 to rabbits, 2ml. per Kg. per day were applied five days a week for 18 weeks without fatalities or any evidence of toxicity. Although a direct comparison cannot be made because of the difference in experimental animal, it was felt that these tests also indicated that the skin absorption of PEG 300 would not be toxic to the rats.

3. Intravenous absorption. Since the flaps of gastric mucosa were hyperemic and bled easily on slight abrasion, intravenous absorption could occur. Rabbits have been given 10 gms. per Kg. of PEG 300 in a single dose intravenously without fatality or toxicity, and 1 gm. per rabbit per day, daily for 5 weeks without any ill effect. From this, it was evident that the intravenous toxicity of PEG 300 is extremely low, so that intravenous absorption through vessels in the flap could not cause toxic reactions.

In summarizing the reasons for the choice of PEG 300 as a vehicle for this work, it was felt that this vehicle satisfied all the demands in that it had the following characteristics:

1. It was a suitable solvent for carcinogenic hydrocarbons.
2. It was non-irritating, so that the rats would not remove it immediately by licking.
3. It was stable and did not hydrolyze or deteriorate on standing.
4. It was completely soluble in water and could, therefore, be absorbed readily by the exposed gastric mucosa.
5. It had been thoroughly tested on laboratory animals over a period of 10 years and was found to be non-toxic in the doses used in this work.

The polyethylene glycol 300 used as a vehicle in this work was donated by Carbide and Carbon Chemicals, Ltd., Montreal.

IX EXPERIMENTAL PROCEDURE

A. THE PREPARATION OF SOLUTIONS FOR PAINTING

1. 0.5 per cent methylcholanthrene solution in polyethylene glycol 300.

0.5 gm. of 20-methylcholanthrene was placed in a 250 cc. Erlenmeyer flask in an oil bath and brought to the melting point of 174-176° C. To this, 100 cc. of polyethylene glycol 300 were added slowly while stirring. The flask was removed from the oil bath and allowed to cool. The 0.5 per cent solution of 20-methylcholanthrene in polyethylene glycol 300 was then stored in a brown glass bottle with a screw top. 10 cc. of this stock solution were poured, when necessary, into a small bown glass bottle having a screw top and used for the painting. The methylcholanthrene used in this work was obtained from Brickman and Company, Montreal.

2. 4 per cent 2-acetylaminofluorene solution in polyethylene glycol 300.

4 gms. of 2-acetylaminofluorene were placed in a 250 cc. Erlenmeyer flask in an oil bath and brought to

the melting point of 191-193° C. To this, 100 cc. of polyethylene glycol 300 were added slowly while stirring. The flask was removed from the oil bath and allowed to cool. The 4 per cent solution of 2-acetylaminofluorene in polyethylene glycol 300 was then stored in a brown glass bottle with a screw top. 10 cc. of this stock solution were poured, when necessary, into a small brown glass bottle having a screw top and used for the painting. The 2-acetylaminofluorene used in this work was obtained from Brickman and Company, Montreal.

B. TECHNIC OF PAINTING

Painting three times a week with carcinogens was first done by Murphy and Sturm in 1925 (144). Mider and Morton (138), in 1939, were probably the first to use 0.5 per cent solutions of methylcholanthrene applied with a No. 6 camel-hair brush. Wilson, De Eds, and Cox (238) first used 4 per cent solutions of 2-acetylaminofluorene for painting.

Rubber gloves were always put on by the assistant who held the rats and by the operator who applied the

carcinogen before handling the animals at any time. The gloves were washed thoroughly with soap and water after each session. This precaution was taken because Gordonoff and Walthard (82) reported the occurrence of "an incipient squamous -cell sarcoma" in the nasolabial fold, a spot often touched by a laboratory assistant who was engaged in applying 0. 3 per cent solution of methylcholanthrene in benzol to the skin of mice.

While the rat was held on its back with the abdomen up, the flap was wiped gently with sterile gauze to remove any secretion, and then painted with one stroke of the brush, so that none of the solution came into contact with the skin. Each paint brush was kept separately with the bottle of carcinogen solution in a labelled container so there would be no possibility of painting with the wrong carcinogen. Painting 3 times a week with the vehicle and the two carcinogenic solutions has been done now for a year, and will continue until all of the animals in the experiment die.

C. THE PREPARATION OF METHYLCHOLANTHRENE IMPREGNATED THREADS.

Threads impregnated with a carcinogen were first used by Andervont (4), in 1936. Howes and de Oliveira (98) used methylcholanthrene impregnated threads to induce adenocarcinoma in the glandular stomach of rats in 1948.

The atraumatic needles with 6 to 8 cms. of 5 zero black silk swedged onto them were saved from the operations. After sterilizing, the ends of these threads were placed in powdered methylcholanthrene, and the beaker containing them was heated over an oil bath until the methylcholanthrene crystallized on them. This procedure was repeated until the threads became yellow and swollen with the carcinogen.

D. INSERTION OF THE METHYLCHOLANTHRENE IMPREGNATED THREADS.

As a trial, a methylcholanthrene impregnated thread was inserted into the submucosa of the flap at operation and cut off flush with the serosa in 6 rats. It was

found, however, that these flaps all shrivelled up within a few days after operation. Thereafter, the thread was not inserted until the tenth post-operative day. By that time the flap had become well established, and the extra trauma of the insertion of a thread impregnated with methylcholanthrene did not damage it. Throughout the experiment, these flaps were wiped gently once a week with gauze and inspected for changes.

It was believed to be unnecessary for this experiment to run a series of animals with untreated silk threads in the flaps or with threads impregnated with an inert substance. Woglom (240), in 1945, tried to cause chronic irritation in the breasts of mice by inserting a black silk thread, but found it caused no noticeable change on microscopic examination. Howes and de Oliveira (98) ran a control series in their experiment, using threads impregnated with a noncarcinogenic compound. They found that no chronic irritation was caused by the presence of these threads in the stomach wall.

E. THE PLAN OF THE EXPERIMENT

By the 10th post-operative day, the skin edges around the flap were completely healed, and the rat was placed in the proper group for treatment.

1. 30 rats were painted 3 times a week with the vehicle, polyethylene glycol 300, only. This group was called the control series.
2. 30 rats were painted 3 times a week with 0.5 per cent methylcholanthrene in polyethylene glycol 300. This group was called the MCA series.
3. 30 rats had a methylcholanthrene impregnated thread inserted into the submucosa of the flap on the 10th post-operative day. This group was called the thread series.
4. 30 rats were painted 3 times a week with 4 per cent 2-acetylaminofluorene in polyethylene glycol 300. This group was called the 2-AAF series.

5. Flaps were prepared on many other rats during the winter of 1950-1951 for later study of the cytologic changes taking place in exposed, but completely untreated, gastric mucosa. 30 of these animals served as an untreated control group.

All of the animals were weighed once a week, and the weights were recorded. It was found that as the flaps hypertrophied and secreted profusely a glarey mucus, pH 8, the animals required more water than could be supplied by the usual 200 cc. water bottle in 24 hours. Consequently, water bottles which could hold 350 cc. were obtained. This supplied an adequate amount of drinking water for 2 animals for 24 hours (Fig. 27).

F. POST-MORTEM EXAMINATIONS AND TISSUE PREPARATION

All cages were inspected twice a day for dead or dying animals. Rats which were moribund were first weighed and then killed with ether. An immediate post-mortem examination was done. Animals found dead were immediately

Fig. 27. Rat cage fitted with large bottles to supply more water to the rats with profusely secreting flaps.

injected with 10 per cent formalin; 10 cc. being placed in the abdominal cavity and 5 cc. into each side of the thorax. A gauze sponge wet in formalin was placed over the flap and held in place with a gauze bandage. The animal was then placed in the refrigerator until the post-mortem examination was done the next day. At this time, the flap was cut out of the abdominal wall with a wide skin margin and then cut in half. One half was preserved in a stock bottle containing 10 per cent formalin , and the other half placed in 10 per cent formalin to be sent for sectioning. The flaps which had had a methylcholanthrene thread inserted were cut in 2 mm. sections at this time, and the entire flap was sent for sectioning. When the abdominal cavity and thorax were opened widely, the lungs, liver, stomach, spleen, and kidneys were removed, and the small and large bowel inspected. Blocks of tissue for sectioning were removed routinely in every animal from the lungs, liver, stomach, spleen, and kidneys and placed in 10 per cent formalin. The remainder of these organs was placed in a separate bottle

containing formalin for later use if more blocks were required. The stomachs were opened along the lesser curvature, washed with water, and examined. When the gastric mucosa had not undergone post-mortem autolysis, a strip 2 mm. wide from the pylorus to the limiting ridge of the forestomach was removed and placed in 10 per cent formalin for sectioning.

Microscopic slides stained with eosin and hematoxylin were prepared of the lungs, liver, spleen, kidney, and flap of every animal. Additional sections of the flaps of every animal were made and stained with Southgate's mucicarmine stain. Serial sections of all flaps which showed unusual regions of glandular growth on examination of the usual routine slides were always done.

CHAPTER III

RESULTS

I GENERAL APPEARANCE AND BEHAVIOR OF THE FLAP ANIMALS

After operation the animals ate well, were sleek, and gained weight steadily at the rate of 2 to 5 gms. a week. The excision of such a large portion of the glandular stomach did not appear to cause any nutritional deficiencies. More drinking water, however, than was normally required for unoperated rats, was consumed by the animals when the flaps be gan to secrete profusely.

This usually began at the end of the second or third month. The abdominal hair was continually wet and became sparse. Many of the animals lost all the hair from the skin around the flaps. This skin, however, remained pink and healthy. The scrotal skin, which was also wet, showed evidence of breaking down where the scrotum rested on the wire mesh floor of the cage, and shallow scrotal ulcers appeared in approximately one third of the animals

in each series. The ulcers would be present for several weeks and then usually heal spontaneously. All scrotal ulcers which were present when an animal died were examined carefully. None penetrated through the full thickness of the skin. On microscopic examination, they were shallow superficial erosions of the epidermis, similar to any decubitus ulcer, infiltrated with inflammatory cells without any evidence of malignancy (Fig. 28).

No incisional hernias occurred in any of the animals, probably because the incision was a left rectus muscle-splitting one, and not the usual midline incision, which results in so many hernias in experimental animals.

Two animals were always kept in each cage, but at no time was there ever any evidence to show that they damaged each other's flaps. Any rat which died during the night would often be found in the morning with its face, legs, or tail gnawed by its cage mate, but the flap of gastric mucosa was never touched.

A severe and unexpected epidemic of pneumonia occurred in the colony of operated animals during the

Fig. 28. Photomicrograph of a "decubitus" ulcer of the scrotum.

(Rat #303. Magnification x 40)

winter, and the death rate rose precipitously (Fig. 34, 35, 37, 39). At first, it was thought that these lung infections were the result of bacteria carried there via the lymphatics from a subclinical peritonitis (128), but post-mortem examination, both gross and microscopic, failed to reveal any evidence of peritoneal infection.

Farris and Griffith (66, p. 519-521) have given a good description of such pneumonia epidemics in many rat colonies. No specific bacteria or virus have ever been isolated. Mucous plugs from bronchiectatic dilatations probably cause atelectasis which in turn leads to pneumonia. Passey, Leese, and Knox (152) have found that bronchiectasis was present in 129 rats in their colony of 251 rats. They stated that this bronchiectasis in rats is in no way related to the bronchiectasis of man. This type of bronchiectasis is similar to Jaagsiekte disease in sheep(24). Inadequate ventilation of the lungs from the forced inactivity of caged animals seems, in their opinion, the most probable cause in rats.

It was impossible to separate the infected rats from the well animals, because definite signs of pneumonia were usually not present until the disease was well advanced. A weight loss of 10 to 15 gms. in one week was often the first evidence of pneumonia. Râles could sometimes be heard, and rapid, noisy breathing was a late sign when the rat crouched in the corner of the cage with its back arched and its hair dry and rough. During January, when most of the deaths occurred, the animals appeared healthy one day, but were found dead the next morning.

At post-mortem examination, the lungs were voluminous and salmon pink in color with large areas of dark red mottling. The cut surface oozed blood, and from the bronchioles, plugs of thick mucoid material or pus could be expressed. Tan colored blebs filled with mucus or shotty caseous nodules were often present in advanced infections.

On microscopic examination, the lungs showed a picture of red hepatization (26, p. 460). The alveoli were packed with red blood cells. All the vessels were large and

filled with blood, and marked peribronchiolar infiltration with inflammatory cells was present (Fig. 29).

The only suggestion that Farris and Griffith (66) have for dealing with such an epidemic is to breed from pneumonia-free animals until a resistant strain is obtained.

II GENERAL APPEARANCE AND GROWTH OF THE FLAPS

At the end of the operation, the flaps generally measured 1.5 cm. in length and 1 cm. in width. The color was good, and there was usually very little bleeding. By the next day, a shiny dark red crust of blood and serum had formed over the flap (Fig. 30). This was not disturbed until 7 days after operation when the sutures were removed. Often this crust had sloughed off spontaneously. If it was still present, it was carefully elevated and removed, leaving healthy looking mucosa beneath it. At this time, the mucosa was 1 to 2 mm. smaller in length and width than at operation, and there were usually small amounts of purulent material between the skin edges and the margin of the flap. This exudate was wiped away with gauze. By the

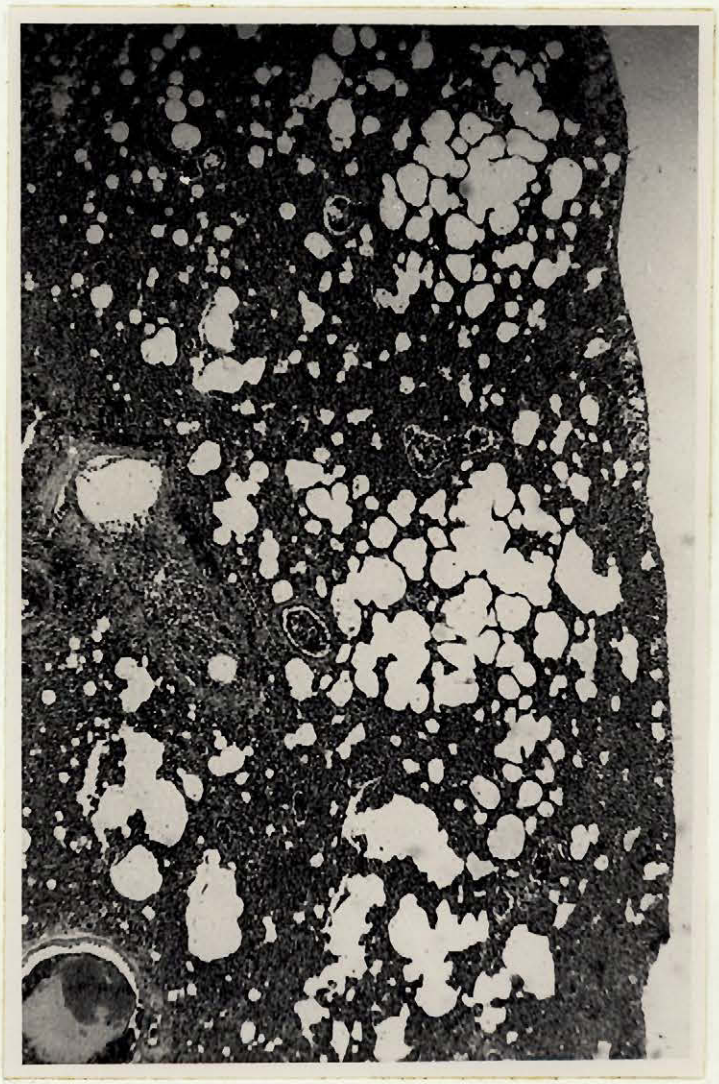

Fig. 29. Photomicrograph showing pneumonia with peribronchiolar infiltration.

Rat #156.

(Magnification X 22)

Fig. 30. Flap on 1st. day after operation showing shiny crust. Rat #344.

tenth day after operation, the skin had grown up tightly around the gastric mucosa, but never grew over it at any time during this experiment.

Occasionally, the edges of the flap became necrotic during the first 10 days after operation, leaving a very small nubbin of mucosa which measured 2 to 3 mm. in length and width (#178). These small portions of mucosa always grew during the experiment, and in several instances measured 1 cm. in width by 2 cm. in length by the time the animal died. This completely unexpected growth potentiality of the gastric mucosa was illustrated to a remarkable degree on two occasions. Rat #282 had no evidence of a flap on careful examination on Oct. 22, five weeks after operation. He was not discarded, but examined each week, and on Nov. 20, a small pink growth of mucosa could be seen in the hair at the site of the former flap. By Dec. 18, this had grown considerably. When he died of pneumonia on Feb. 14, the flap measured 1 cm. in length and 0.5 cm. in width. The second instance of this regenerative power was seen in rat #275. Three months after operation,

on Dec. 15, this rat caught his flap on the sharp edge of a feeding box and tore it off. There was a severe hemorrhage, and his weight dropped from 200 gms. to 166 gms. during that week. When the crust of blood was removed from the site of the flap on Dec. 18, the left rectus muscle was exposed, and no mucosa was visible. By Jan 3, just 19 days after the accident, a definite flap of gastric mucosa was present. When this animal died of pneumonia on May 22, five months after the accident, the flap measured 1.3 cms. in length and 1.2 cms. in width. In both instances, small remnants of mucosa, not visible on gross examination, must have been present, and from these the mucosa was able to regenerate.

A biopsy of the flap was done on three rats (#127, #186, #220). This was accomplished easily with a sharp scalpel, but the bleeding was so profuse that several sutures had to be put in to stop the hemorrhage. These areas became infected, and after one animal died from the infection (#127), it was decided to abandon this procedure.

After 5 to 6 weeks most of the flaps grew considerably thicker, and in many instances definite buds of mucosa protruded from the surface and at the margins of the flaps. These areas always bled very easily (Fig. 31 & 32). This was a reaction to chronic irritation of the exposed gastric mucosa. "Tom", the patient with a gastrostomy, studied by Wolf and Wolff (241, p. 10) had these buds of hypertrophied mucosa grow on the edge of his gastrostomy opening when it was constantly irritated by a gauze dressing as he did heavy labor with a pick.

There were wide ranges in color change in these flaps. The blanching due to fright, which Drury, Florey, and Florey (54) found in the patch of colon mucosa in the dog, could be easily demonstrated. If a rat struggled violently when held in an uncomfortable position, the flap became a bright red, due to the increased hyperemia. This hyperemia is similar to the observations made by Wolf and Wolff (241, p. 162) in their human subject. The normal color of the flaps was the deep pink of healthy gastric

Fig. 31. Hypertrophied flap of rat #161. Weight 355 gms.

Date of operation: July 19th. 1950.

Date of photograph: May 21st. 1950.

The mucosa has been exposed 10 months and has been painted 3 times a week with polyethylene glycol 300.

Fig. 32. Side view of flap shown in Fig. 31.

mucosa. Rats which had râles, and those dying of pneumonia, always had cyanotic flaps.

The flaps secreted varying amounts of mucus. Often it would be only a thin transparent film. At other times, thick white opaque flakes would be adherent to the flaps. Occasionally, a single, large, clear drop of mucus could be seen collecting and dripping from the flap as the animal slept in a corner of the cage. This mucus was always alkaline, just as Wolf and Wolff (241, p. 163) found. No special tests were done on the secretion at this time, because a complete study of the secretion, using the new Bollman apparatus (23) for holding a rat, has been planned for next year.

III HISTOLOGY OF THE RAT'S STOMACH

Before discussing the gross and microscopic findings in each series, a brief description of the histology of the rat's stomach has been included, because it varies in several aspects from the histology of the human stomach. Berg (18) and Shay (184) have both given excellent histological descriptions which were a great help in this work.

The forestomach (rumen) is lined by stratified squamous epithelium, which is thickened and elevated along the limiting ridge. Here an abrupt change from squamous cells to columnar cells occurs. The submucosa in this region is composed of dense connective tissue, often containing glandular acini.

The glandular portion of the stomach is divided into a fundus and an antrum. The mucosa of the fundus consists of long, narrow, tubular glands lined by numerous large, round parietal cells, and smaller chief cells. Near the mid-portion of the glands are several large cells which show mucin granules when stained by an appropriate technic. The transition between the histological pattern of the mucosa of the fundus, which is thrown up in rugae, and that of the antral mucosa is gradual and may be compared to the intermediate zone of Aschoff as described by Babkin (8). The glands of the antrum are shorter and wider than those in the fundus and are lined by columnar epithelium. Brunner's glands often extend from the intestine up into the antrum beneath the muscularis mucosae for a short distance

above the pylorus. In the antrum, round, lymphoid nodules or diffuse patches of lymphoid tissue often occur (Fig. 33).

The glands of the stomach are supported by a loose connective tissue containing a few lymphocytes. Beneath this connective tissue is a well-developed muscularis mucosae which is separated from the true muscularis externa by another thin layer of connective tissue. Beyond this is a thin serosa.

This normal histological picture is present in flaps placed in formalin on the day of operation and prepared for sectioning. Flaps which have been growing for five weeks on the anterior abdominal wall without any treatment present an entirely different picture. These sections were reviewed by Dr. Catherine Stevens of the Department of Histology, and her comment was as follows:

"On the day of operation the histology is normal. Five or more weeks after operation the usual distribution of cells in this part of the stomach is entirely replaced by cells resembling those of the surface epithelium. Some sort

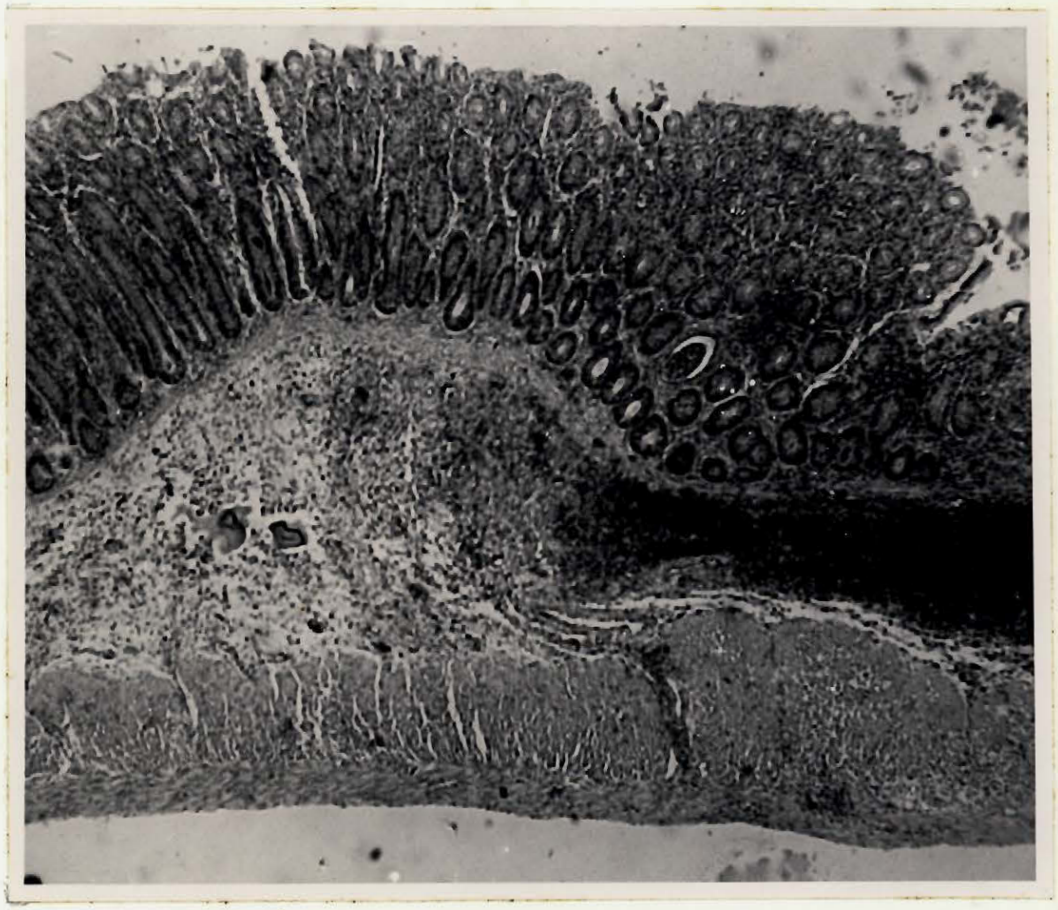

Fig. 33. Normal gastric mucosa of the rat showing lymphoid tissue in the submucosa.

(Magnification X 22).

of deep glandular arrangement is retained, parts appearing cystic. Probably there is a general pyknosis and degeneration of all the cell types originally present (very likely the thickness of the mucosa is much reduced). In the normal gastric mucosa the only cell type which undergoes mitotic division is the surface mucus type. This cell formation occurs at the base of the gastric pits and balances a loss of cells by extrusion from the surface of the epithelium. Thus, when there is a general destruction of the mucosa, probably the only cell capable of division is the surface mucus type; this type would then proliferate and replace those which disintegrate. The resulting epithelium would then be of the purely surface mucus type as observed."

The results are presented according to the plan of the experiment as described on p. 85.

IV RESULTS IN THE CONTROL SERIES

These 30 rats were painted 3 times a week with the vehicle, polyethylene glycol 300. Six rats are still

alive and have good flaps which will be painted until these animals die. One animal was discarded and killed after being in the experiment for 3 months, because the flap disappeared. The rest had good flaps at the time of death, except rat #241, which tore the flap at the end of 6 months. An abscess formed and perforated into the liver, killing the animal. Six animals in this series had scrotal ulcers. The number of deaths monthly is shown in Fig. 34.

Causes of Death in the Control Series

30 animals in the series
6 animals still alive
24 died during the experiment

18 died of pneumonia
2 died of bronchiectatic abscesses
2 died of liver abscesses from infected flaps
1 died of pericarditis after cardiac puncture
1 died, killed with ether, no flap after 3 months

Microscopic examination of the flaps in the control series

Three flaps (#154, #170, #186) of this series presented a picture of cystic hyperplasia. These cystic areas were situated in the mucosa and in the submucosa

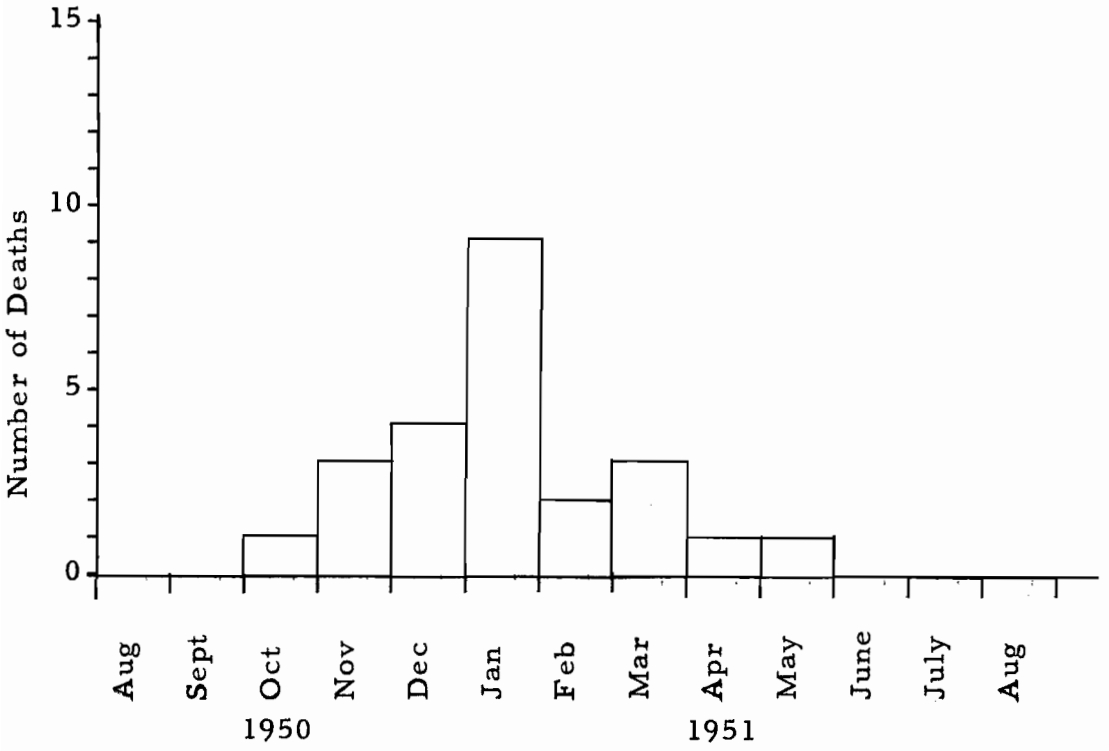

Fig. 34. Chart showing the number of deaths monthly in the control series painted with polyethylene glycol 300.

The smaller cysts were lined by columnar or cuboidal cells containing mucin granules. The larger cysts were lined by flattened cells also containing mucin granules. The amorphous material in many of the small cysts stained for mucin with mucicarmine. Several areas of intracystic papillary projections were present on each slide. Many of the surface glands were widely dilated, and all contained mucin granules. There were no inflammatory cells present.

The flap of rat #186 was biopsied on Dec. 8, 1950. This showed a "Swiss-cheese" cystic hyperplasia. The flap, removed when the animal died on Jan. 3, 1951, had an identical structure. Three other flaps of this series (#156, #172, #178) showed several small and large cysts widely scattered among normal glandular patterns. On 6 other flaps (#157, #160, #178, #188, #192) deep islands of glandular cells were found in the submucosa. The cells all contained mucin granules and, in most instances, formed small acini, The remaining flaps of this series

showed vessels packed with red cells, more so than were present in normal gastric mucosa. None of the flaps in this series had osteoid tissue or adenoms.

V RESULTS IN THE MCA SERIES

These 30 rats were painted 3 times a week with 0.5 per cent methylcholanthrene in polyethylene glycol 300. Seven rats are still alive and will be painted until they die. One animal was discarded after being in the experiment one month because the flap disappeared. The remaining animals had good flaps at the time of death. Nine animals in this series had scrotal ulcers. The number of deaths monthly is shown in Fig. 35.

Causes of Death in the MCA Series

30 animals in series
7 animals still alive
23 animals died during the experiment

21 died of pneumonia
1 died of liver abscesses
1 discarded, no flap after 1 month

Microscopic examination of the flaps in the MCA series

A biopsy of the flap of rat #220, which is still alive, showed definite cystic hyperplasia. Again in this

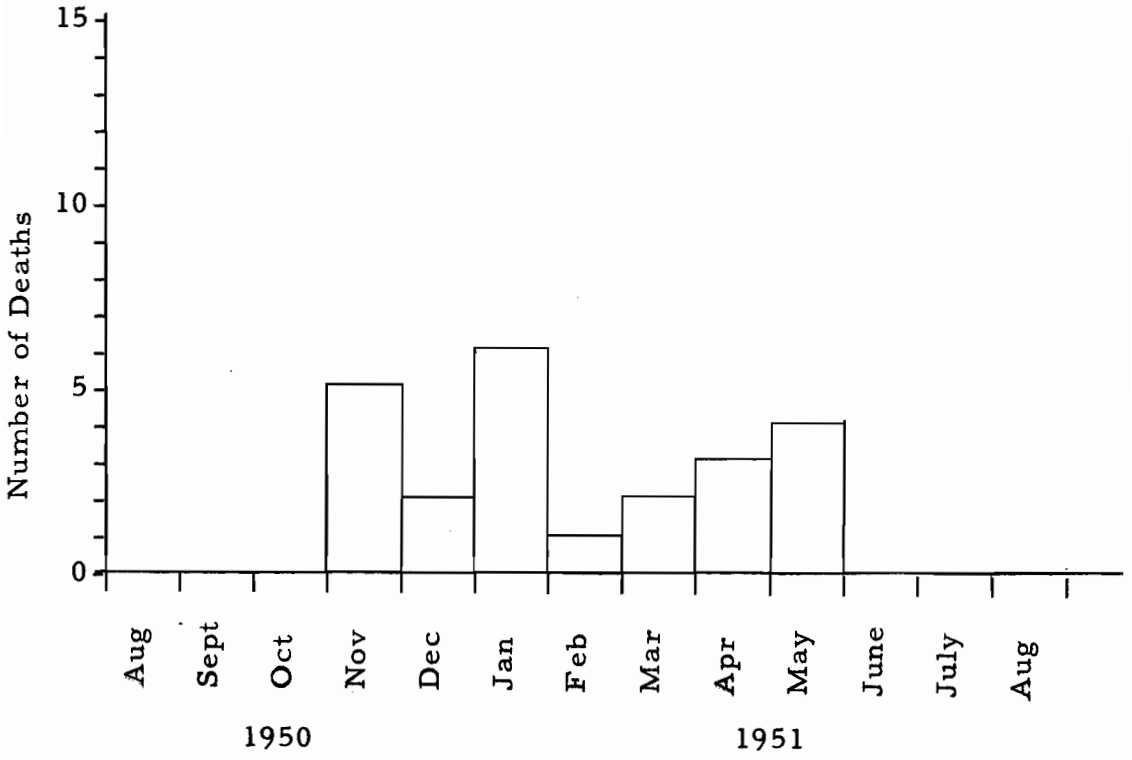

Fig. 35. Chart showing the number of deaths monthly in the MCA series.

series, 3 flaps (#200, #206, #222) showed cystic hyperplasia with the same histologic picture as previously described (Fig. 36 & 49).

In this series, 7 flaps (#208, #210, #217, #221, #226, #228, #233) had adenomas. (The flap of rat #208 is shown in Fig. 42.). Deep nests of mucin containing gland cells were found between the strands of the muscularis externa. In some areas these cells retained a definite glandular pattern; other areas showed only acini, while more wild areas were merely collections of compressed mucin containing cells without any pattern. Non of these areas had perforated through the flap to the anterior abdominal wall. One flap, #213, showed strands of mucin containing cells lying between muscle bundles of the muscularis externa, as well as acini (Fig. 43).

The remaining flaps in this series showed gastric mucosa with slightly dilated glands.

One rather unusual finding, in the flap of rat #217 of this series, was several small areas of osteoid tissue lying between the gastric glands (Fig. 47). Since these areas were discovered in the flaps of other series, they will be discussed later.

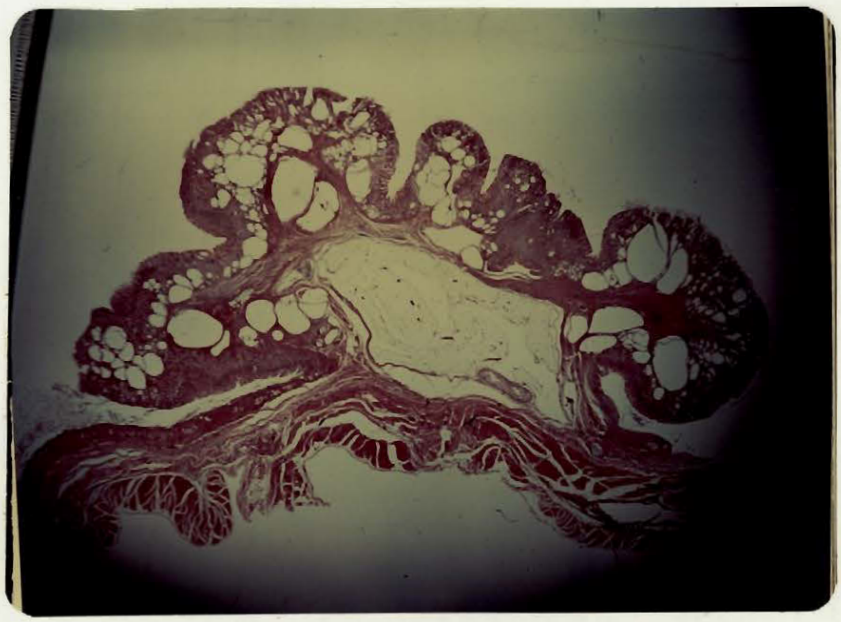

Fig. 36. Photomicrograph of flap showing cystic hyperplasia.

Rat #206, MCA series.

Operation: August 4th. 1950.

Died: March 2nd. 1951, from pneumonia.

(Magnification X 40).

VI RESULTS IN THE MCA THREAD SERIES

These 30 rats had a methylcholanthrene impregnated thread inserted beneath the gastric mucosa of the flap 10 days after operation. Eight rats are still alive and will be observed until they die. Two rats (#291, #298) were discarded after being in the series for 2 months, because the flap disappeared. One rat died at the end of 4 months with lung and liver abscesses. The remaining deaths, 19, in this series, were all due to pneumonia. Five animals in this series had scrotal ulcers. The number of deaths monthly is shown in Fig. 37.

Causes of Death in the MCA thread Series

30 animals in series
8 animals still alive
22 animals died

19 died of pneumonia
1 died of lung and liver abscesses
2 discarded, no flap

Microscopic examination of the flaps in the MCA thread series

Small areas of cystic hyperplasia were present in only 3 flaps (#282, #300, #303) in this series. No threads were found in any of the flaps at post-mortem

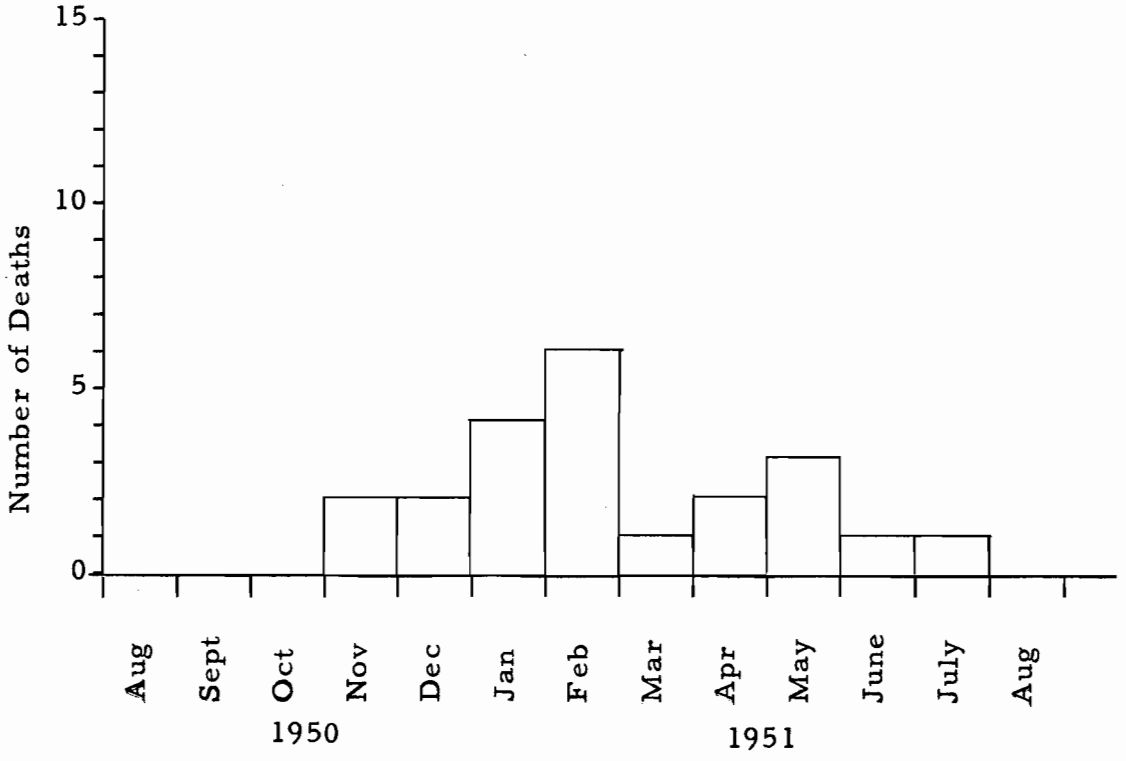

Fig. 37. Chart showing the number of deaths monthly in the MCA thread series.

examination or on sectioning for the slides. One flap (#297) showed a sinus tract where the thread had been. This is shown in Fig. 38. The normal glandular pattern was disturbed and concentrated around the tract. All these cells contained mucin granules. Twelve of the flaps revealed adenomas (#271, #272, #275, #277, #279, #282, #292, #293, #295, #297, #308, #309). These adenomas were in the muscularis externa and varied from small collections of mucin-containing round cells without any pattern (#272, Fig. 40), through areas containing glandular cells arranged in small acini (#277, Fig. 41), to adenomas with a complete glandular pattern (#271). All of these adenomas were circumscribed and had not infiltrated the anterior abdominal wall.

Osteoid tissue was found between the glands in 6 flaps (#275, #279, #297, #302, #308, #309). The remaining flaps in this series showed gastric mucosa with slightly dilated glands, and, in a few instances, small papillary projections into the dilated glands.

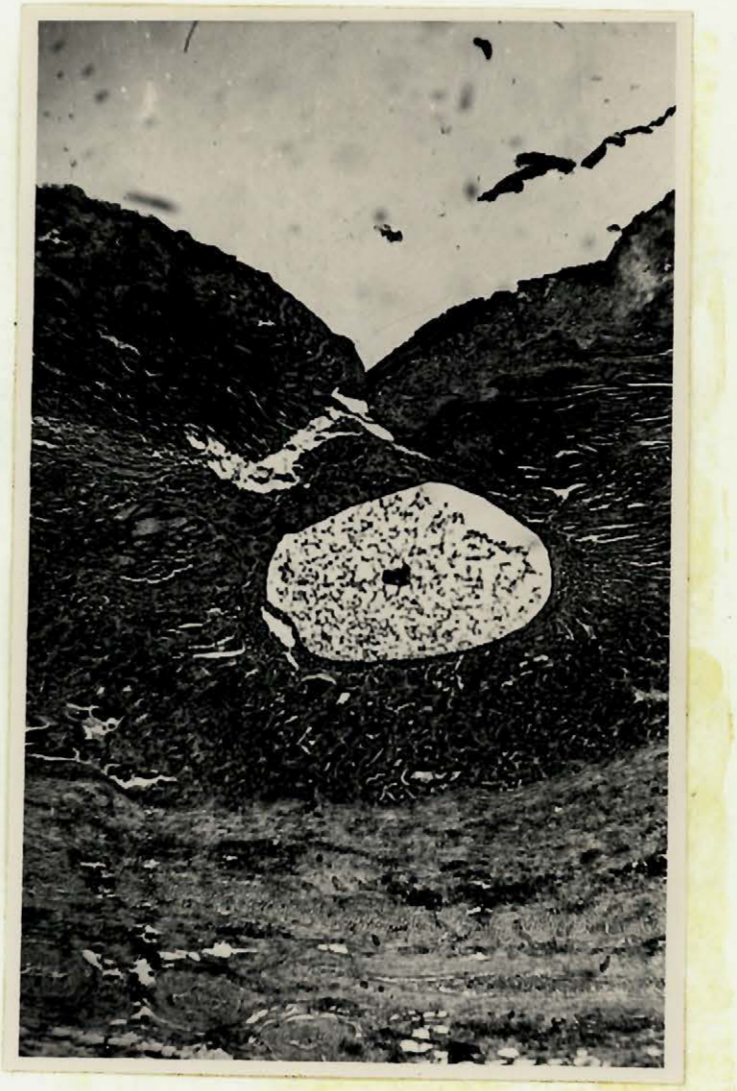

Fig. 38. Photomicrograph of flap, showing a sinus tract where the M C A thread had been.

Rat #297, MCA thread series

Operation: Sept. 26, 1950.

Died: Feb. 16, 1951, from pneumonia.

(Magnification X 22).

VII RESULTS IN THE 2-AAF SERIES

These 30 rats were painted 3 times a week with 4 per cent 2-acetylaminofluorene in polyethylene glycol 300. Eight rats are still alive and will be painted until they die. No rats were discarded after the series started. Two animals died of unusual causes. One rat (#249) had 18 small bladder stones at post-mortem examination. A small stone was wedged in the urethra and must have acted as a ball-valve for a long period before causing complete obstruction, because the bladder was thick walled, and filled nearly the entire abdominal cavity. The other animal (#238) tore his flap on the cage and licked the oozing blood until he had exsanguinated himself. All the organs were blanched at post-mortem examination, and the entire intestinal tract was dilated with blood in various stages of digestion. No distant tumors or hepatomas have been found in these rats. Seven animals in this series had scrotal ulcers. The number of deaths monthly is shown in Fig. 39.

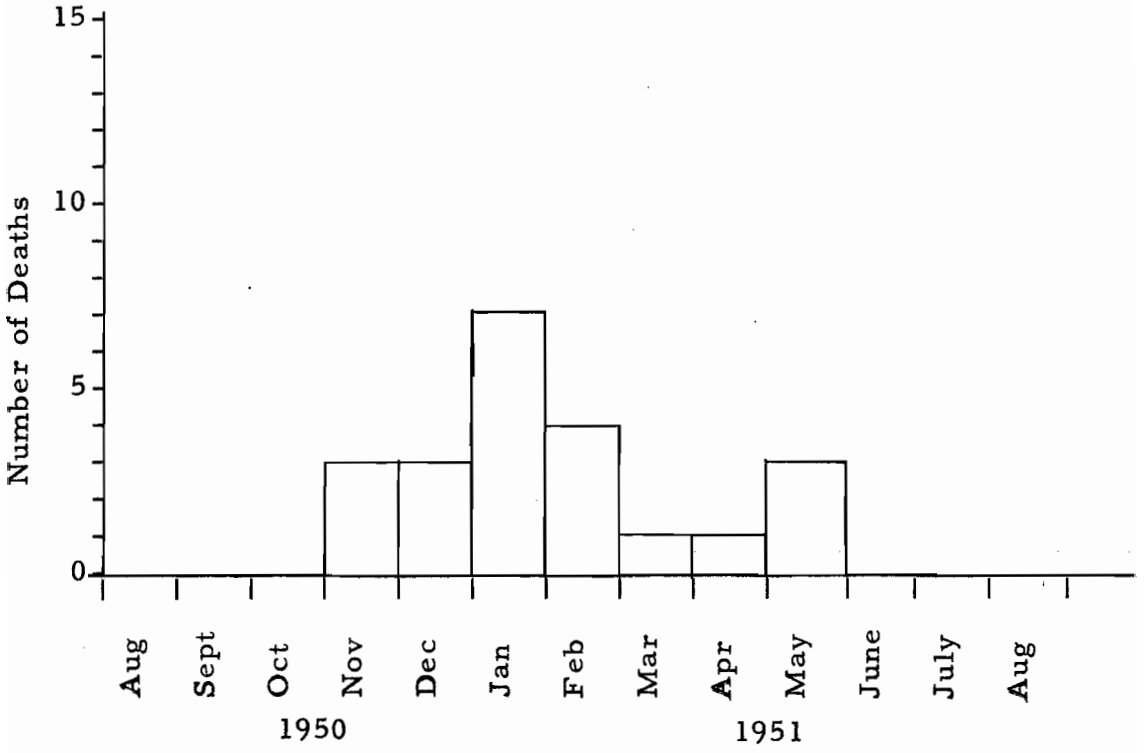

Fig. 39. Chart showing the number of deaths monthly in the 2-AAF series.

Causes of Death in the 2-AAF Series

30 animals in the series
8 animals still alive
22 animals died during the experiment

18 died of pneumonia
1 died of liver abscesses
1 died of bronchiectatic abscesses
1 died of acute urinary retention
1 died of hemorrhage

Microscopic examination of the flaps of the 2-AAF series

Two flaps in this series (#257, #259) showed the cystic hyperplasia previously described. In this series, 3 flaps (#253, #270, #288) had several adenomas located in the muscularis externa. The surface blood vessels in all of the flaps of this series were widely dilated, and, in many areas, the surface layer of the mucosa was replaced by amorphous material. This was found only in the 2-AAF series. Six flaps of this series (#174, #238, #247, #253, #261, #265) showed small scattered areas of osteoid tissue.

VIII RESULTS IN THE UNTREATED CONTROL SERIES

These 30 rats had flaps of gastric mucosa which were completely untreated. None of the flaps disappeared after the animals were placed in the series. Five animals have died in this series to date. One rat (#324) died after being in the series for 3 months from acute urinary retention caused by obstruction of the urethra with a bladder stone. Four animals died of pneumonia (#321, #325, #334, #342). At the post-mortem examination, one rat (#321) had several shallow scrotal ulcers. The remaining 25 rats have no scrotal ulcers and are healthy. These animals do not appear to have any nutritional deficiencies, or to be harmed in any way by having a flap of gastric mucosa placed on the anterior abdominal wall. It is too early yet to determine whether the life span has been shortened by the operative procedure.

Microscopic examination of the flaps in the untreated control series

Cystic hyperplasia to the degree shown in Fig. 36 was present in one flap (#325). The other four flaps showed no cystic areas. No osteoid areas and no adenomas were found in these flaps. The glandular pattern and mucus-secreting cells were similar in all cases to those found in the control series treated with the vehicle, polyethylene glycol 300.

IX SUMMARY OF THE MICROSCOPIC EXAMINATION OF THE FLAPS:

CYSTIC HYPERPLASIA was present in:

1.	Untreated control series	1	flap	out	of	the	5 examined.
2.	Control series with PEG 300	3	"	"	"	"	24 "
3.	MCA series	4	"	"	"	"	23 "
4.	MCA thread series	3	"	"	"	"	22 "
5.	2-AAF series	2	"	"	"	"	22 "

ADENOMAS were present in:

1.	Untreated control series	0	flap	out	of	the	5 examined.
2.	Control series with PEG 300	0	"	"	"	"	24 "
3.	MCA series	7	"	"	"	"	23 "
4.	MCA thread series	12	"	"	"	"	22 "
5.	2-AAF series	3	"	"	"	"	22 "

OSTEOID TISSUE was present in:

1.	Untreated control series	0	flap	out	of	the	5 examined.
2.	Control series with PEG 300	0	"	"	"	"	24 "
3.	MCA series	1	"	"	"	"	23 "
4.	MCA thread series	6	"	"	"	"	22 "
5.	2-AAF series	6	"	"	"	"	22 "

X SUMMARY OF THE ADENOMAS found on microscopic examination of the flaps of rats dying during the experiment from various causes.

MCA SERIES

Number of months treated	Number of flaps examined	Number of flaps with adenomas
3	1	0
4	5	0
5	3	1
6	4	2
7	2	0
8	0	0
9	3	3
10	4	1

MCA THREAD SERIES

Number of months treated	Number of flaps examined	Number of flaps with adenomas
3	3	0
4	4	2
5	5	4
6	2	0
7	0	0
8	3	3
9	2	2
10	1	1

2-AAF SERIES

Number of months treated	Number of flaps examined	Number of flaps with adenomas
3	2	0
4	4	0
5	3	1
6	6	1
7	1	1
8	3	0
9	1	0
10	0	0

XI DISCUSSION OF RESULTS

It must be clearly understood that the gastric neoplasms obtained to date in this work are not gastric cancers, because they do not meet all the criteria established by Klein and Palmer (108), in 1941, which are:

1. The ability to proliferate independently as metastases.
2. The ability to invade progressively and destructively neighboring tissues and organs.
3. Irreversibility of the properties in the absence of the extrinsic factor initially held responsible for the cellular change.
4. Reasonable evidence to indicate a causal relationship of the experimental procedure to the tumor (108,p. 582).

These tumors, which lie in the submucosa and in the muscularis externa, are definite adenomas of the stomach conforming to Herbut's definition of: "Tumors composed of epithelial cells in glandular formation supported by scanty stroma of loose connective tissue." (88, p. 348) He also stated that : From the morphologic viewpoint, gastric adenoma and papilloma are known to be precancerous lesions . . . ." (88, p. 350)

The adenomas, found in the flaps, are always well circumscribed, but the cell pattern within them varies greatly. Some of the adenomas have a regular glandular pattern, but many have small and irregular acini. A few adenomas are merely collections of glandular cells without any pattern. However, in every case, the cells contain mucin granules, which stain a bright pink with Southgate's mucicarmine stain. (Fig. 40, 41, 42, 43)

Howes and de Oliveira (98) obtained a number of gastric adenomas in the rat with methylcholanthrene impregnated threads and decided that adenomas developed if the carcinogen had acted for only a short period, in some instances for only 2 months. In the flaps, no adenomas were found until the carcinogen had acted for at least 4 months. Howes and de Oliveira stated that when the carcinogen acted for longer periods (exactly how long, they neglected to say), the reticulin barrier surrounding the adenoma was destroyed, and these mucus-secreting cells broke through the normal connective tissue barrier, infiltrating adjacent organs and metastasizing to distant organs.

Fig. 40. Photomicrograph of adenoma showing small irregular acini.

Rat #272, MCA thread series.
Operated: Aug. 22, 1950.
Died: June 11, 1951 from pneumonia.
(Magnification x 22).

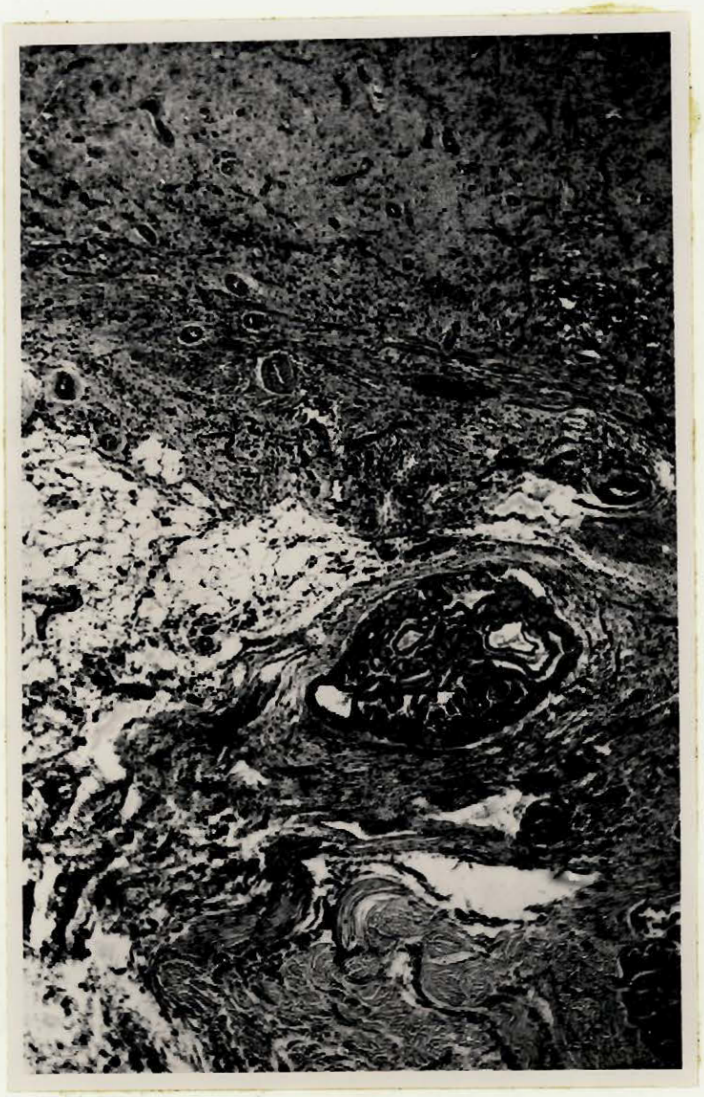

Fig. 41. Photomicrograph of adenoma showing irregular glandular pattern.

Rat #277, MCA thread series.
Operated: Aug. 22, 1950.
Died: Dec. 16, 1950 from pneumonia.
(Magnification x 22)

Fig. 42. Photomicrograph of glandular areas between muscle fibers.

Rat #208, MCA series.
Operated: Aug. 7, 1950.
Died: April 28, 1951, from pneumonia.
(Magnification x 22)

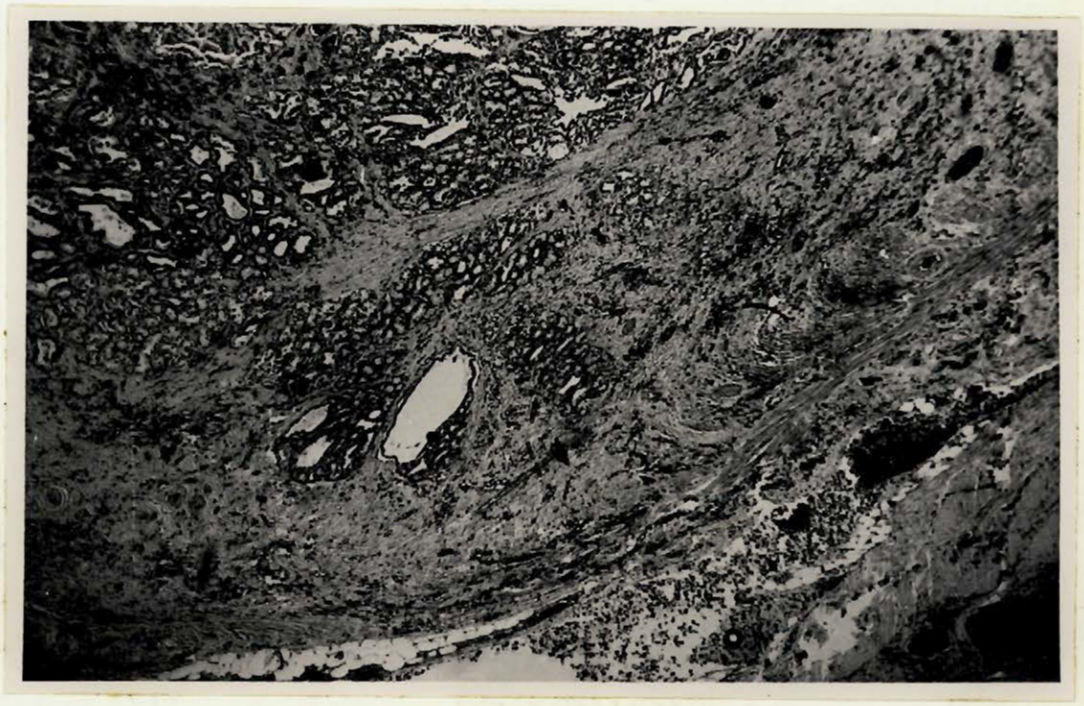

Fig. 43. Photomicrograph of acini and cystic areas between muscle fibers.

Rat # 213, MCA series.
Operated: Aug. 8, 1950.
Died: May 21, 1951, from pneumonia.
(Magnification x 22)

Both Cantero (35) and Stewart (212) have found that the minimum period for the development of gastric adenocarcinomas in the rat is 12 months. Consequently, there is a good possibility that when the animals in this experiment have been treated for more than a year, gastric cancer will be found.

It was very difficult to decide early in the experiment reported here, whether the adenomas obtained in the flaps were due to the carcinogen, or whether they were produced simply by chronic irritation. Stewart and Lorenz (214) obtained the adenomas shown in Fig. 44 by injecting methylcholanthrene into the stomach wall of mice. An identical picture, however, was obtained by Bullock and Rohdenburg (32) when they introduced spine balls into the stomach cavity of rats (Fig. 45). Adenomas were never found in the untreated flaps, nor in those treated only with the vehicle. It appears that the chronic irritation of exposure alone will not produce adenomas in the flaps. Treatment with a carcinogen seems necessary for their production.

FIGURE 5.—Adenomas of the stomach from three mice. *A*, Lesion is markedly atypical, and except for the fact that it has not infiltrated through the muscularis to the peritoneum, it is structurally indistinguishable from other lesions which had permeated the gastric wall and were classified as adenocarcinoma. × 27; *B*, Lesion is better differentiated than in *A*, but it has extended down to the muscularis. × 27; *C*, Muscularis mucosae is destroyed, and the submucosal nodule is composed of atypical glands which rest on the muscularis. × 39.

Fig. 44. Photomicrograph from Stewart and Lorenz (214, p. 183).

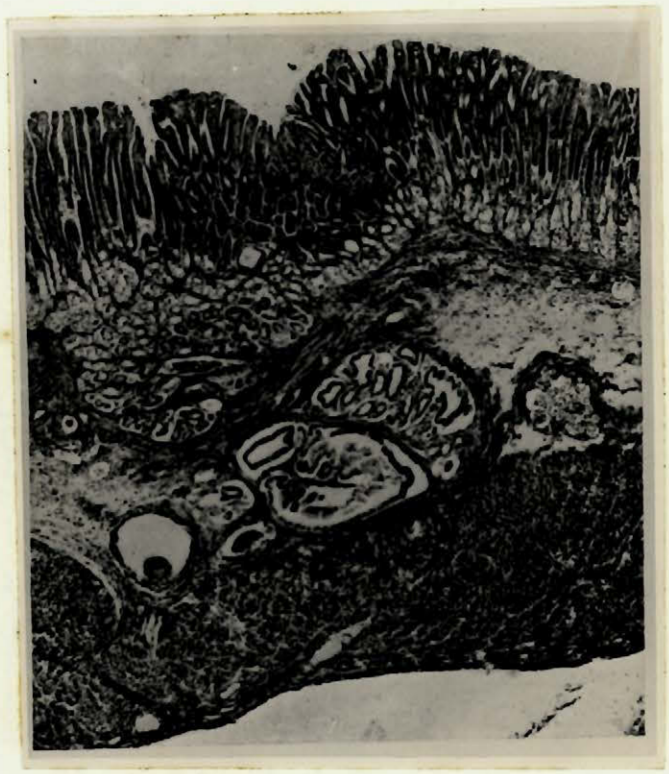

Fig. 45. Photomicrograph from Bullock and Rohdenburg (32, p. 247).
"Healed ulcers, (ulcer produced by spine ball) of stomach resembling cystadenoma, showing various types."

In view of the fact that Howes (96) has stated: "The mucous cell is the only cell capable of growing into a gastric neoplasm." (96, p. 384) It is very fortunate that the only cell of the normal gastric mucosa of the rat which persists after transplantation of the flap to the anterior abdominal wall is the mucous cell. Stout (219) has also stated that: "Almost all gastric carcinomas are derived from the mucus-secreting cells of the stomach." (219, p. 809) (Fig. 46)

It is well known that the gastric glands contain four major types of secretory cells, but many different names have been proposed for them, so that the nomenclature is rarely alike in any two descriptions (129, p. 384). Babkin's description (8), with additions from Maximow (129) and Hollander (94), is given here in order to form a basis for the discussion of cell changes which have been found to occur in exposed, damaged, or diseased gastric mucosa.

1. Mucous cells, which secrete a viscous mucus, are also called mucous neck cells, chief cells of the neck, mucoid cells, Nebenzellen and Zwischenzellen, or cellules principales muqueuses.

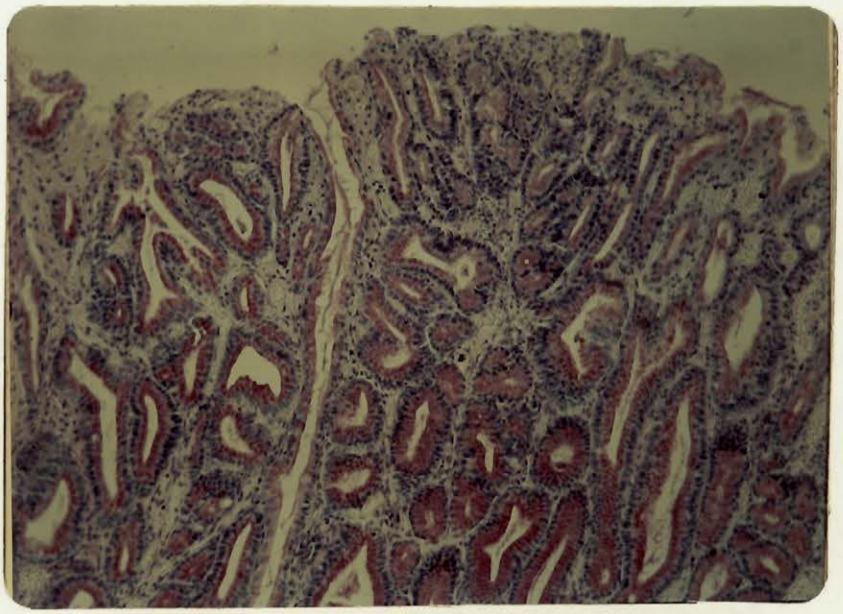

Fig. 46. Photomicrograph showing mucin granules in all the gland cells.

Rat #178. Control series, PEG 300.
Operated: July 26, 1950.
Died: Jan. 26, 1951, from pneumonia.
(Southgate's mucicarmine stain.)
(Magnification x 180)

2. Peptic cells, which secrete the enzymes, are also known as body chief cells, chief cells of the body, zymogenic cells, central or adelomorphous cells.

3. The parietal cells, which contribute the HCl, are also known as acid cells, oxyntic cells, delomorphous cells, and Belegzellen.

4. The fourth type of cell is called argentaffine cells of Heidenhain by Maximow (129). Hollander (94) calls his fourth type, mucous cells of the surface epithelium and omits argentaffine cells; whereas Babkin (8) calls the fourth type merely surface-epithelium cells.

Ferguson (67) studied the regeneration of gastric mucosa in the dog following surgical removal of small rectangles of mucosa. He stated that: "Evidence indicates that the specialized cells of the gastric gland are capable of passing through a cycle of transformation followed by regeneration." (67, p. 433) This is a very controversial subject, but the reverse process, that is, transformation of the specialized cells enumerated above into mucus-secreting cells of the surface epithelium type is stated by Babkin(8)

to be fairly well established. It has been found to occur in gastritis, gastric ulcer, and cancer by Konjetzny (112). Florey and Harding (77) transplanted "patches" of fundic stomach to the abdominal wall of the cat and found that: "One of the reactions of fundal mucosa to prolonged irritation is to differentiate, with the production of mucus-containing cells." (77, p. 451) It seems that Popoff's observations more nearly coincide with the process taking place in the flaps when he stated that: "Pyloric and cardiac mucus-secreting glands show cytomorphosis of the rejuvenation type, this being especially pronounced in the stomach of the rat." (160, p. 883)

From the observations made by Dr. Catherine Stevens on the microscopic sections of the flaps of gastric mucosa, it appears very doubtful that either a "transformation" or a "differentiation" of the specialized cells of the gastric mucosa occurs. It seems more probable, in her opinion, that during the first few weeks of exposure there is a general pyknosis and degeneration of all the cell types originally present in the gastric mucosa. Then the

mucous cells of the surface epithelium proliferate and replace those which disintegrate, so that after a few weeks of exposure on the anterior abdominal wall, all of the glands are composed of mucus-secreting cells of the surface epithelium type.

The mucus secreted by normal gastric mucosa acts as a protective barrier in man and animals. To a certain extent, it protects the mucosa from physical injuries caused by ingested substances, and also from auto-digestion by the gastric juices. In experimental animals, it appears to protect the stomach from the action of orally administered carcinogens (12, 99). It is for this reason that so many experiments have been devised to try to destroy this mucus-barrier (95, 204, 113). By having the flaps of gastric mucosa exposed on the anterior abdominal wall where the mucus can be easily removed by wiping the flap with dry gauze before applying the carcinogen, there is no longer a mucus-barrier to prevent the absorption of the carcinogen.

The flaps, by their position, are exposed to chronic irritation, so that when osteoid tissue was discovered lying between the gastric gland in many microscopic sctions, it was first believed that chronic irritation alone was the cause. This belief was supported by the studies of Bullock and Rohdenburg (32), in 1918, who found that the rat's stomach, irritated by introducing a spine ball into the lumen, reacted by forming osteoid tissue. They stated: "In or bordering the area of injury, small areas of connective tissue of the stratum proprium are sometimes converted into osteoid tissue." (32, p. 232) No osteoid tissue, however, has so far been found in any of the flaps of the control series in this work. Only in those flaps which have been treated with carcinogens, has osteoid tissue appeared (Fig. 47). Dr. Harold L. Stewart, Senior Pathologist of the National Cancer Institute at Bethesda, believes these areas of osteoid tissue are of significance in the flaps(213). He has found similar areas of what he prefers to call "areas of osteogenesis" in the 4 gastric adenocarcinomas which he recently obtained in a series of 265 rats injected beneath

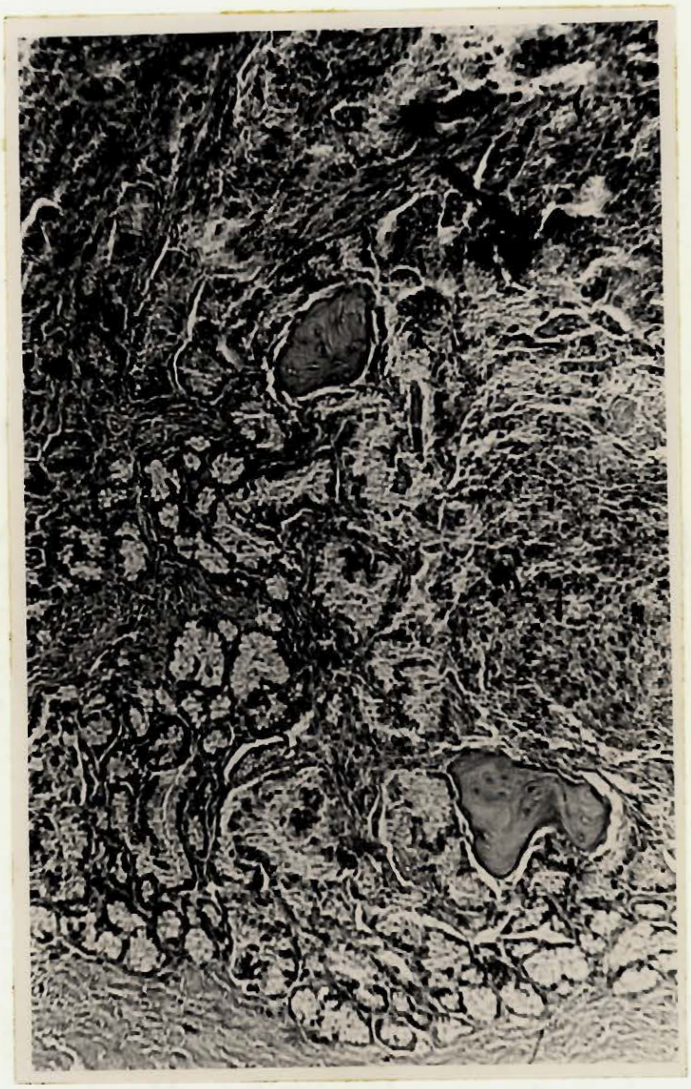

Fig. 47. Photomicrograph showing two areas of osteoid tissue lying between gastric glands of flap.

Rat #217, MCA series.
Operated: Aug. 8, 1950.
Died: Jan. 27, 1951, from pneumonia.
(Magnification x 100)

the gastric mucosa with suspensions of methylcholanthrene in horse serum. He believes these areas arise from the irritation of the carcinogen.

Hyperplasia of the mucosa is evident in all of the flaps(Fig. 48), and, in some flaps, cystic hyperplasia is present to such a great degree that they resemble polypoid growths (Fig. 49). However, it is quite clear, after careful study of the sections under the microscope, that this is not a hypertrophic gastritis, because there is no infiltration with inflammatory cells. Wolf and Wolff (241) were confronted with the same decision in their studies of the gastric mucosa through "Tom's" gastrostomy opening. They decided that although the folds were thick, red, and succulent, and bled easily, thereby assuming all the characteristics of what is known to the gastroscopists as "hypertrophic gastritis", this term could not be "properly applied, since there was no indication that actual infiltration of inflammatory cells occurred." (241, p. 162) Stout (219) stated that: "The criteria of what constitutes gastritis are not established, but vary according to the personal prejudices of different observers." (219, p. 807)

Fig. 48. Photomicrograph of flap showing the normal hyperplasia.

Rat #270, 2-AAF series
Operated: Aug. 21, 1950
Died: Feb. 14, 1951, from pneumonia.

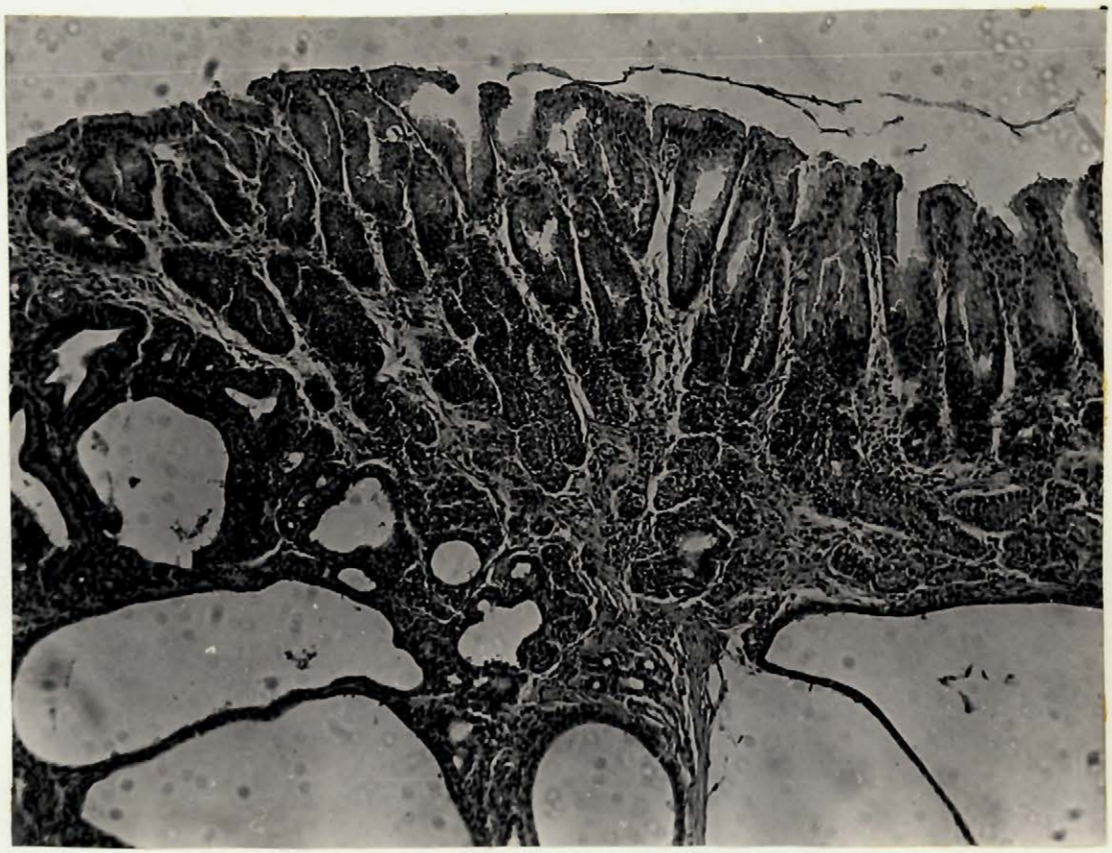

Fig. 49. Photomicrograph showing detail of hyperplasia.

Rat #206, MCA series.
Operated: Aug. 4, 1950
Died: March 2, 1951, from pneumonia.
(Magnification x 100)

This can be readily confirmed by reading the definitions of chronic hypertrophic gastritis and chronic atrophic gastritis in such textbooks of pathology as Boyd (26), Karsner (103), Herbut (88), and Schafer (168).

The replacement of the normal cells of the gastric glands by mucous cells, which occurs consistently in these flaps, should probably be designated as a type of chronic atrophic gastritis. Ivy (99) has stated that when the peptic and parietal cells of the gastric mucosa are replaced by mucous cells: "This is the type of change that is seen in atrophic gastritis and in the region of some gastric ulcers and cancers and of gastroenterostomies." (99, p. 319) Chronic atrophic gastritis may be of many different varieties, according to Cox (43), and the only point of agreement in all of the varied definitions is that mucus-secreting cells alone form the gastric mucosa. This cell change may be very important in the eventual production of gastric carcinoma in these flaps.

In attempts to produce experimental cancers, irritating substances, such as croton oil and turpentine, and such procedures as scarifying and burning the skin, are known as promoting agents. They have often been used to stimulate tissues before the actual carcinogens have been applied. Shubik (192) has tried a great number of these substances on the skin of rats, and found that all were ineffectual. The reparative hyperplasia of wound healing, however, was a successful promoting agent in the skin of rats, according to Kline and Rusch (110). Berenblum (17) has also stated that hyperplasia is an essential precursor of neoplasia, but that preneoplastic hyperplasia may be of a different biological type than the usual reparative hyperplasia. Peacock has stated that: "Even when dealing with potent chemical carcinogens, like benzpyrene, we have ample evidence that the mere presence of the hydrocarbon in contact with the tissue will not necessarily lead to tumour growth; normal resting tissues do not react to carcinogens unless their equilibrium is in some way

disturbed." (153, p. 135) It is felt that the reparative hyperplasia which takes place after the operation and during the healing of small abrasions sustained by the flaps, may act as a promoting agent in this experiment.

In summary, it has been found in this experiment that adenomas appear only in flaps treated with carcinogens for at least 4 months. A cell change occurs from the exposure of the gastric mucosa, leaving only mucus-secreting cells, which are considered by some authorities as the only cell capable of growing into a gastric cancer. The mucus-barrier, which has deterred the absorption of carcinogens administered orally, can be removed from the flaps with gauze. Osteoid tissue has formed only in flaps treated with carcinogens. In spite of the hypertrophy which is present to some degree in all flaps, the changes in the gastric mucosa of the flaps, caused by exposure, appear to be those of chronic atrophic gastritis. The reparative hyperplasia, which takes place in the flaps, may act as a promoting agent. All of these factors point to the development of gastric adenocarcinoma when sufficient time has elapsed.

CHAPTER IV

CONCLUSIONS

1. A successful operating technic has been devised for the transplantation of flaps of gastric mucosa to the anterior abdominal wall of the rat. The death rate and morbidity are low. The flaps grow well, and the animals suffer no apparent disability from having the flaps on the anterior abdominal wall.

2. A vehicle, for dissolving carcinogenic hydrocarbons, has been found to be of value as a non-toxic, water-soluble substance absorbed readily by the gastric mucosa.

3. The transplanted mucosa undergoes changes from the exposure, which are compatible with a description of chronic atrophic gastritis. Mucus-secreting cells replace the normal gastric mucosa. Both of these changes are conducive to the formation of gastric adenocarcinoma.

4. After treatment with carcinogens, adenomas, which are considered precancerous lesions, develop in the flaps. It is believed that if the flaps are treated for more than a year in this manner, gastric adenocarcinoma can be expected to occur.

CHAPTER V

SUMMARY

The object of this work was to devise a technic for exposing the gastric mucosa of the rat so the carcinogens could be applied locally to cause the development of gastric adenocarcinoma, which then could be watched step by step.

The literature on clinical and experimental gastric adenocarcinoma has been reviewed with the purpose of finding all of the extrinsic and intrinsic factors which may lead to the growth of gastric cancer. With these factors in mind, a new vehicle for the two carcinogens, especially chosen for this work, was used. The literature on the vehicle and on the carcinogens has been reviewed.

Experimental operating technics for the exposure of gastric mucosa have also been reviewed, and the technic devised for this work has been described in detail.

Rats with flaps of gastric mucosa were divided into 5 series, each of which comprised 30 animals. Two series were used as controls, two series were treated with methylcholanthrene, and one series was treated with 2-acetylaminofluorene. The results of the post-mortem examinations and the examinations of the microscopic sections of the flaps have been summarized.

Adenomas, which have been considered precancerous lesions, have been obtained in each of the series treated with carcinogens.

Reasons for expecting that the materials and methods used in this experiment will produce gastric adenocarcinomas in the surviving animals, after a sufficient period of treatment, have been given.

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