* CARCINOMA OF SMALL INTESTINE

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A. CARCINOMA OF SMALL INTESTINE

I. INTRODUCTION

Carcinoma of small intestine is of rare occurrence. In the Mayo Clinic, the total number of carcinoma of gastro-intestinal tract recorded up to the year 1918 was 3,555 and of these only 24 cases were found in small intestine; 5 in duodenum, 11 in jejunum 2 in appendix and 6 in ileum, while of the rest, 1,689 were found in stomach and 1,822 in large intestine. The most frequent site was the large bowel, including rectum being 52.3 per cent, the next frequent in stomach 47 per cent, and the small intestine only 0.67 per cent.

According to the report of Nothangel, who has studied the result of autopsies performed in the city hospital of Vienna during twenty five years, the number of cancers found in various parts of the body was 4,494 (8.5%) of a total of 53,152 autopsies and of these cases 446 (9.9%) were found in intestine. Of these 446 intestinal cancers 441 (95.7%) were in large intestine while only 25 cases (4.3%) were found in small intestine. Another striking figure can be cited from the report of Forgue and Chavin, who discovered 6,347 (8%) cases of carcinoma in series of 88,031 autopsies. Of these cases of carcinoma 642 (9.2%) were found in intestine, of which 613 (95%) were in large bowel leaving only 29 (5%) in small intestine. Based on these reliable records, it can be roughly estimated that the incidence of carcinoma found in autopsy is about 8 per cent, and the same findings in intestine about 9.5 per cent, and the occurrence of carcinoma in small intestine about 5 per cent of intestinal tract or 0.5 per cent of all carcinoma.

The German statistics of carcinoma of intestine were about 8.56 per cent and the United States census of 1914 revealed it to be 11.8 per cent. It is very remarkable to note that 95 per cent of carcinoma of intestine is found in the large bowel and the remaining 5 per cent may be distributed on various portions of the small intestine despite the fact that its length is about five times as great as that of the large intestine. Tumours of the small intestine of any kind are rare, and the most frequent tumour is lymphoblastoma. According to a study of cases made by Heng Liew, it occurs about three times as often as either adenoma or carcinoma which rank second in frequency to tumours of the small intestine.

The rarity of carcinoma, as well as other forms of tumour in the small intestine, has been noted by various authors and many attempted explanations have been made to solve the underlying factors, some of which are as follow: — (1) The liquid nature of the contents of small intestine saves the organ from trauma. (2) The abundant pancreatic

secretion present in small intestine makes the development of tumours difficult. (3) Parasitic sterility. Though no satisfactory explanation can account for the rareness of tumour in small intestine, as a matter of fact the cause of tumour itself is yet to be found. The fluidity of intestinal content and its alkalinity, and its passage through the long intestinal tract in comparatively short time, seem to play an important role in preventing the mucous membrane of intestine from either chemical or mechanical irritation, both of which are noted to be important factors either directly or indirectly in the development of carcinoma.

It has been stated that the incidence of carcinoma of small intestine increases as we approach either end of the gut; near the stomach proximally and near the colon distally, carcinoma at the middle portion of the small intestine is extremely rare. This singular fact seems to support the view that the small intestine is well protected from both chemical and mechanical irritation, thus making the development of carcinoma difficult in this region. In the proximal and distal portions however, where the protection is less complete, carcinoma develop in greater frequency.

According to Ewing, the carcinoma of small intestine assume three distinct forms, the tumour would either be annular, polypoid or nodular. The annular form indicates an early metastasis around the wall which causes constriction of the lumen of intestine, the polypoid form, which is rather rare in small intestine and more frequent in large intestine, is usually a pedunculated mass of neoplastic tissue either single or multiple. This polypoid form has been moulded to its shape by peristaltic movement of intestine and often causes complete obstruction of the intestinal canal. The nodular form is seen in the case of the so-called carcinoid tumour or in early stage of polypoid form, either causing no interference if it is too small, or partial and persistent obstruction if it is of sufficiently large size to narrow the lumen of the intestine.

It has been stated by Mayo that carcinoma of small intestine, when developed, is usually engrafted upon a polyp; an adenoma or a papilloma. Whether the malignant tumours develop secondary to benign ones, or they are primarily malignant at the beginning, cannot be determined. However, the most common type of carcinoma throughout the gastro-intestinal tract is found to be adenocarcinoma originating from glands of Lieberkuhn, and the next most frequent type is colloid carcinoma, which may result either from adenocarcinoma or from papillary carcinoma derived from the epithelium of the mucous membrane of the intestine which undergoes colloid degeneration after invading the surrounding tissue. Generally speaking, carcinoma of the small intestine seem to fall into two categories; adenocarcinoma and carcinoid tumour which is really benign in many

respects. In regard to the status of the latter tumour much work has been done lately and it seems necessary to exclude it from the group of carcinoma and place it in a new category of tumour. The detailed description of the tumour and some of the recent investigations on this particular tumour will be presented in the latter part of this paper separately under carcinoid tumour.

The symptoms of carcinoma of small intestine may vary according to the localization, the size and the form of the tumour.

The primary and the essential symptoms are local ones due to mechanical disturbance wrought by constriction or obstruction of the passage of intestinal contents, which would cause either partial, intermittent or complete obstruction of the lumen. The duration may also vary from a few years to a few days depending on the rate of growth and the form of the tumour causing the interference, for instance in the constricting form early symptoms are likely to appear constantly, whereas in the polypoid form the symptoms may be sudden complete obstruction, but often intermittent in nature. The localization of the tumour on the long range of intestinal tract apparently results in producing symptoms of slightly varying degree.

The duodenum is a very frequent site for malignancy considered on figures presented in statistics and also in comparison with the rest of the small intestine in regard to their respective lengths. Jefferson found on his study of 71 collected cases of carcinoma of small intestine, 34 cases were in duodenum while the remaining 37 cases were in the rest of the small intestine. In the Mayo Clinic report by Judd in 1919, 5 out of 24 cases of carcinoma of small intestines were in duodenum which corresponds to only 20 per cent. While another report by Kohler found 9 duodenal cancers in a total of 12 cases of carcinoma of small intestine which is 75 per cent. In view of the above contradictory figures, it seems unwise to make any statement on the incidence of carcinoma of duodenum. However, the incidence of carcinoma of duodenum seems to be far greater than the rest of the small intestine as judged by their respective lengths. Ewing gives an original figure reported by Ciesers for the incidence of duodenal cancer as about 4 per cent of intestinal carcinoma.

Duodenal cancer has been divided into three distinct groups. Periampullary, supra-ampullary and infra-ampullary, based on the anatomical situation and also on symptomological basis. The ampulla upon which the common duct opens into the intestine seems to be a more frequent site for carcinoma than any of the remaining portions of the duodenum. According to some authors 70 per cent of duodenal cancers occur in this region, around which is the meeting place of intestine with biliary and pancrea-

tic ducts, and the head of the pancreas is closely in contact with the outer layer of the duodenum. The next most frequent seat is on the supra-ampullary region and the infra-ampullary region is the least frequent site. The ampullary or peri-ampullary region of the duodenum is an apt site for tumour growth and even very small tumours in this region excite marked symptoms at an early stage, peculier to its anatomical and physiological character, namely, an early jaundice accompanied by rapid emaciation. Though the incidence of cancer in this region may be high, yet a determination of the original tissue from which the tumour may have arisen is not an easy task, due to crowding of many tissues into a small area and fusion of one with another. The supra-ampullary region which is an area between ampulla and pylorus of stomach is stated to be next frequent site of duodenum. The previous existence of ulcer in this region of duodenum, has been blamed by many authors for the frequent incidence of carcinoma. Jefferson had collected 30 cases from the literature in which malignant change had been suspected from duodenal ulcer. Judd has reported, in the collected papers of Mayo Clinic in 1910, 5 cases of duodenal cancer in this region and he found at least two cases showed history of previous ulcers, while the rest of the cases had no evidence of pre-existing ulceration at this region. Although it is generally believed that about 10 per cent of gastric cancer may have arisen from previous ulcers, yet there seems to be no adequate evidence to show that all duodenal cancers originate from pre-existing ulcers. However, the more frequent discovery of duodenal ulcers in recent years, may in future throw more light on this subject, which at present is a matter requiring careful study. A very illustrative case was reported by Jefferson in which a cancer of duodenum originated from an ulcer obstructing Wirsung's duct. Judd after studying 5 cases of duodenal carcinoma stated that papillary tumour, which so readily becomes malignant, is one of the most common type of carcinoma of the first portion of the duodenum, often the papillary tissue seems at times to extend across the pylorus into the stomach. Infra-ampullary cancer of duodenum is very rare and only a few cases have been reported in the literature. The symptoms are said to resemble those of pyloric obstruction, except that there is a greater amount of bile mixed with the vomitus and it shows the presence of pancreatic juice.

Carcinoma of jejunum is more frequently found at the site of junction between jejunum and duodenum. However, some 10 to 15 cm. below this junction is found to be a more favourable site for development of carcinoma. The carcinoma of jejunum and ileum may arise either as a ring form of carcinoma, or as a degenerating polyp. and the former seems to be more frequent than the latter especially in the jejunum. Most of

the 11 cases of carcinoma of jejunum reported by Judd were this type.

Cancer of ileum, like cancer of jejunum, occours as an annular constricting or the ring type, only a few cases have been found as polypous or papillomatous growths. The symptoms are similar to obstruction of jejunum; severe colicky pain and signs of intermittent obstruction, but no definite localization of pain can be detected as a possible site for obstruction. In six cases of carcinoma of ileum studied by Judd, 4 cases had symptoms of short duration, being present from one to twenty one months. In 1 case there had been indefinite intestinal symptoms for ten years and in this particular instance the lesion was found as a degenerating polyp of ileum. It is said to be impossible to determine the location of the tumour from the clinical history, and in most instances a general diagnosis of intestinal obstruction was made, just as in the case of the tumours of the jejunum. Carcinoma of the ileo-caesal valve is extremely rare and often it occurs from the carcinoma of adjoining ileum extending into the valve.

The appendix is a very noted seat of malignancy. MacCarty discussing malignancy of the appendix stated that in a series of 2,000 specimens 0.6 per cent, or about 1 in every 176 appendices removed at operation, were found malignant microscopically, and all of them were without clinical symptoms. In another series he described, about 0.44 per cent of removed appendices were found to be malignant which is 1 in every 225. In a very large percentage of these appendices, where malignancy was observed, either partial or complete obliteration of lumen with marked inflammation was present. Carcinoma of appendix was found in a child of five years and also in a case at the age of eighty. The first report of carcinoma of appendix was made by Clazobrook in 1895, who described a large tumour as big as a pigeon's egg. According to Ewing carcinoma of appendix are either adenocarcinoma or carcinoid tumour and most of the primary carcinoma of appendix are of the so-called carcinoid type which is a tumour of specific entity. Shortly after the discovery of primary carcinoma of appendix many more reports followed and later it was found that the peculiar type of tumour which had been described under the term primary carcinoma of appendix, also occurs in jejunum and ileum which is similar to that already described by Lubarsch in 1888. Carcinoma of appendix or carcinoid tumour of appendix are remarkable in that they occur in about 2.5 per cent of all partially or completely obliterated appendices and in about 0.6 per cent of all appendices removed. Thus, the symptoms of carcinoma of small intestine though they vary to a certain extent as to its localization, size and its form, as described above in different parts of the small intestine, yet the primary and principal

symptoms are the same throughout; namely partial, complete or intermittent obstruction of intestinal canal. Diagnosis is difficult in most cases, due to failure of the pain to localize the lesion and difficulty in detecting a mass in its early stage. Secondary symptoms or the general symptoms are of comparatively late appearance, i.e. cachexia due to malignant growth in any part of the body. The patient usually succumbs to symptoms of intestinal obstruction before any suggestion of possible malignancy could be made clinically, and further even though some definite mass could have been palpated in the abdomen at an early stage, there seems to be no definite signs which can be attributed to the presence of carcinoma, instead of some other more frequent tumour of intestine. In polypoid forms of cancer, a few cases of intususception were reported having the upper portion of intestine invaginated into a lower portion in passing through an unmovable portion of gut due to the presence of a tumour. In regard to the symptoms of the nodular form of growth, which are mostly carcinoid tumours no clinical symptoms have been observed and these are all found upon autopsies performed for quite another disease.

The number of tumour masses found in intestine are usually single but they often occur as multiple growths and if they are found to be multiple, they are usually considered to have been engrafted on original benign polyps which occur frequently throughout the intestinal tract. The carcinoid tumour seems to occur in the multiple form as a rule, but they will be discussed later.

The metastases are usually limited to mesenteric lymph nodules and a wide metastasis which is usually the case in gastric cancer in the liver lungs etc. is rare in the case of intestinal carcinoma. It may be partially due to the fact that the small mass of new growth in the intestinal tract would usually produce comparatively earlier and more serious symptoms than in the case of carcinoma of stomach, before any marked metastases occur. It is interesting to note that Mayo has made a statement, describing about 50 per cent of all the cases of intestinal carcinoma which showed no lymphatic involvement at autopsy. As to the histogenesis of carcinoma of intestine, we naturally look for the cells akin to the tumour cells in normal histology of small intestine, since it is an established fact from the time of Virchow that, "only similar cells reproduce similar cells" in either normal or pathological conditions.

The epithelial cells of small intestine are derived originally from the embryonical endodermal layer and they are constantly replaced by basal cells at middle portion of Lieberkuhn's glands. In stomach the replacing cells are found active at the neck of the gland, while in intestine the mitotic figures are numerous further down in the middle portion of the

glands and they replace the cells on both sides, the epithelium above and glandular cells below.

The carcinomatous transformation of cells can either come from the epithelial layer of the mucosa, or from the glandular cells of the glands of Lieberkuhn. The former origin is seen in the cases of polypoid carcinoma or papillary carcinoma where the new growth projects into the lumen, while in most of the annular or ring form of carcinoma, the tumour instead of forming a definite mass, early invades the surrounding tissue and apparently originates in the cells of the glands of Lieberkuhn. These cells are seen assuming the formation of glandular tissue in rather crude fashion due to their intrinsic character wherein they are destined to gland formation in normal state as well as in pathological conditions. In regard to histogenesis of cancer of duodenum, some suggest that they may have arisen from the glands of Brunner and though this may be possible, still their actual occurrence has been considered doubtful by many authors. Another view advanced was that carcinoma may arise from accessory pancreatic rests which are often found on intestinal wall, especially in duodenum and jejunum. Still another is the theory of implanted epithelial tissue in submucosa which may survive for an indefinite time in a state of latency only to develop malignancy if the proper opportunity is offered to the tissue. Ewing and others suggest that adenocarcinoma may develop from the benign carcinoma or carcinoid tumour of intestine, though no actual evidence was as yet drawn to prove the possibility. These views and theories are interesting for they are all possible factors in development of carcinoma of small intestine. However the carcinoma of small intestine, in the majority of cases, is thought to develop from ordinary existing columnar or cuboidal celled epithelium, covering the normal mucosa and forming the ducts of the glands of Lieberkuhn.

The age and sex incidence of intestinal carcinoma is not of any special value, since both sexes are equally disposed to the malignancy and the age incidence seems to occur in similar proportion in both sexes as they are found elsewhere in the body. According to the United States census of 1914, on the mortality due to carcinoma, it was stated that among cancer deaths of 52,420, 6,234 cases (11.8%) were found in peritoneum, intestine and rectum, of which 2,690 (43%) were in male and 3,544 (57%) were in female, showing a slightly higher incidence in female sex. In the collected papers of the Mayo Clinic of 1918, Judd reported a very remarkable account of 11 cases of carcinoma of jejunum in which 6 were in male and 5 in female, with an average age of 46 years ranging from 34 to 65 years old. In another of his series of carcinoma of duodenum in 5 cases the average age was 53 years. The earliest incidence of in-

testinal carcinoma was recorded by Duncan in a child of 5 years and the latest age incidence was between 60 and 65 years. Ewing states the average age as 46½ years in his book on Neoplastic Diseases.

II. THE MATERIAL AND THE TECHNIQUE

The writer's present study of carcinoma of small intestine is based on a case of typical carcinoma found in the jejunum, and three cases of carcinoid tumour two in ileum and one in appendix. Special care was exercised for the comparative study of carcinoma and carcinoid tumours. All the materials were fixed either in formalin or Zenker's fluid according to their need, and after paraffin embedding and sectioning, various stains were employed; haematoxylin eosin, Van Gieson, Sudan iii for frozen sections, silver staining method of Hasegawa and also special Giesma staining was done at the suggestion of Professor Robinson who has given me many invaluable aids in this paper.

CARCINOMA OF JEJUNUM

Autpsy No. 154, 1927, Department of Pathology, University of Toronto. Patient——male age 65.

Brief clinical history: The patient had pain and discomfort of three years duration coming on after meals in the upper abdomen, and a gradual loss of weight and sight. Increasing constipation was noted for one year and mucous stools for a few weeks before admission. Two days before admission signs of complete obstruction developed, with crampy pains and bile stained vomitus. On physical examination the stomach was distended and tympanitic, the abdomen moved slightly with respiration, and general abdominal tenderness with muscular rigidity was present. A firm palpable mass was found in the left upper quadrant. The mass was thick but no visible peristaltic movements were observed. Laboratory work,—W.B.C. 19,200, 93 per cent polymorphonuclear cells, R.B.C. 3.5 million, haemaglobin 61 per cent.

A diagnosis of intestinal obstruction and carcinoma of splenic plexure of colon was made. At operation a large amount of greenish yellow vile smelling purulent material was found in the abdomen. The abdomen was closed with drainage, a week later the patient's temperature rose and he died ten days after admission to the hospital.

AUTOPSY

The small intestine was found with a firm mass causing a constriction that entirely encircled the bowel at a point about 15 c.m. distal to the duodeno-jejunal junction. The mesentery at this point was considerably thickened and indurated.

The tumour was excised and preserved for museum specimen, and it consisted of a portion of intestine measuring about 10 c.m. in length with a portion of the mesentery broadly attached to the intestine which measured about 12 c.m. in diameter.

The intestine was firmly constricted by rather strong elastic band of about 2 c.m. in thickness which was found completely encircling the lumen of the intestine leaving a very narrow passage for an object not larger than a lead pencil. At the base of this band was an ulceration, which measured about 2 c.m. in diameter. The floor of this ulcer was firm and nodular in appearance, and this firm irregular base was found to be the edge of a spherical mass in the mesentery which was firmly attached to the intestine. The mass of tumour which occupied the mesentery adjacent to the intestine and the edge of which forms the base of the ulcer described, was a spherical mass of tissue measuring about 6 c.m. in diameter with a thickness of about 4 c.m. It was firm and elastic on palpation and dull greyish in colour. Speckles of yellow tinge which appeared to be the remnants of mesenteric fat, were found throughout the mass. The central portion of the tumour mass was necrosed and crumbled on touching, and a cavity measuring 5×2.5 c.m. was found in this necrosed area. The wall of the cavity was filthy in appearance and tinged with green pigment apparently from bile. At one corner of this cavity a minute sinus was found leading a proble into the base of the ulcer in the intestine and it seems evident that the bile, as well as other contents of small intestine, had leaked out into abdominal cavity through this sinus with resultant general peritonitis. The cavity of the tumour mass seems to have resulted from the action of pancreatic enzyme on the necrosed portion of the tumour. The margin of this mass was semi-circular in appearance and giving the impression that the tumour was spreading in a wavelike manner. The mesenteric lymph glands were not palpably enlarged and no suspected metastasis was found either in the liver or in any other part of the body.

III. MICROSCOPICAL OBSERVATIONS

Sections were made from various portions of the tumour, at the base of the ulcer, from the fibrous constricting band encircling the wall of the intestine and from the tumourous mass in the mesentery. The sections showed that the muscular coats were somewhat thickened and the submucosa was considerably fibrosed. In the mucosa, rugae stood out very prominently, but the covering epithelium was entirely lost throughout. The glands of Lieberkuhn are, however, well preserved with numerous globlet cells mixed with the long cells and the paneth cells at the base

of the acini, each had inclusions of mucous droplets and slightly acidophilic granules respectively. The blood vessels are considerably engorged throughout and lymphatic infiltration was marked throughout the intestinal wall. The mucosa became suddenly thickened near the margin of the ulceration in which were found atypical cell masses forming gland-like structures, which produced a striking contrast to the normal glands of Lieberkuhn in the immediate neighbourhood. On higher magnification these atypical cells are found to be stained with nuclei of varying sizes and shapes; some were spherical, oval, or elongated in their shape, beside showing irregularity in their size. The nuclei stained quite deeply with haematoxylin and contained unequal numerous chromatin granules within the nuclear membrane, which also stained irregularly, not infrequently in a beaded appearance. The cytoplasm was also deeply stained with eosin, and the amount of it was fairly abundant in these cells. The shape of the cells was not uniform in appearance, some losing their columnar shape and shifting into a cuboidal or spherical contour but they are all arranged in a somewhat irregular glandular structure. The cells are packed together and dispose themselves into single or multiple layers forming acini of varying shapes and sizes which contain in many instances cells of similar types in their lumina. In some acini these cells are clumped together with pyknosed fragments of nuclear substance. No mucous formation was observed in any of these alveoli, but mitotic figures were frequently encountered. On going deeper along the margin of the ulceration, more glandular tissue was observed among the normal appearing glands of Lieberkuhn, until one reached a portion where the normal arrangement of the mucosa was completely destroyed by the presence of more massive alveoli, irregular both in size and arrangement of glands, the stroma became scanty and was replaced by a very narrow strip of fibrous connective tissue infiltrated with lymphocytes. The cells begin to assume a more irregular shape, size and in arrangement of alveoli formation. The larger the alveoli, the more necrosed cells are found in the central portion of the alveoli. A general impression is that in some places the small glandular arrangements fused together leaving traces of irregular lumina of glands, while on the other hand masses of cells were packed together in clumps without lumina. These pseudo-glandular masses of all sizes were found invading downward, destroying the submucosa and the muscular layer, and replacing them. Near the base of the ulcer along the margin, the muscular coats are entirely destroyed and fragments of muscular coats are seen between and at each end of the massive tumour which consists of irregular alveoli surrounded by a delicate fibrous stroma. At the base of the ulcer, the transformation of tissue was so complete that no trace of normal tissue was visible. The

structure merely consists of irregular masses of alveoli bound together by a fair amount of fibrous connective tissues which are infiltrated by lymphocytes and a small number of endothelial cells and polymorphonuclear leucocytes. The structure of the base of the ulcer is continuous with the main mass of tumour embedded in the mesentery, no boundary line between the intestine and mesentery could be made out either macroscopically or by microscopic study. The small alveoli are found usually shrunken, leaving spaces between alveoli and stroma. In very small alveoli, the shrunken spaces were seen to be covered hy flattened endothelial cells reminding one of enormously dilated lymph channels. The portion of tissue which caused constriction of the intestine was next examined. It was found to be made up of strands of adenomatous masses of atypical cells similar in character to those found in the other part of the tumour, the alveoli are separated irregularly by abundant strands of fibrous connective tissue. This structure was observed in one part just between what appeared to be compressed muscular coats and normal mucosa and in another place it was found to replace entirely the normal tissue and stretched out from the lumen to the outer coat of the small intestine. The fibrous connective tissue was more abundant in the section made from the mass in the mesentery, which may be termed scirrhous carcinoma differing slightly from other parts only in the amount of fibrous connective tissue in the stroma.

IV. DISCUSSION

The tumour cell nests, which are composed of atypical epithelial cells forming gland-like arrangements, have been observed throughout, beginning at the mucosa where in one portion they are found in contact with normal gland and in another place entirely replacing the normal glands. The cells were found in a mass gradually invading the muscular coat, and at the base of the ulcer, an area was found with an entire disappearance of muscular coats and fusion into the mass found in the mesentery. In short the impression obtained from various parts of the tumour is essentially the same, varying only in the matter of degree of fibrous connective tissue contained in the stroma of tumour. Around the ulcer was abundant alveolar structure with scanty stroma which may be called " medullary ", but the band of tissue constricting the wall of intestine and the main mass of tumour found in the mesentery was tumourous tissue which contains more abundant connective tissue fibres, but with comparatively scanty alveolar structure, which may on the other hand be designated as " scirrhous carcinoma ". However, be it medullary or scirrhous, the essential structure of adenocarcinoma was the same throughout, differing only in the proportions of tissue they contained.

In the clinical history the patient complained of three years of pain and discomfort in the upper abdomen and increased constipation for a year. This history does not necessarily indicate that the patient has had carcinoma for three years, as the growth in intestine may have started either as an adenoma or papilloma which though benign in nature, nevertheless gives similar intestinal symptoms. However, the increased constipation may have been due to gradual constriction of the intestinal wall, caused by infiltration of carcinoma as a band around the lumen of the intestine, and in this case may have taken a year which may be considered of comparatively slow growth. The abundance of fibrous connective tissue in the stroma, which renders the substance of tumour to feel firm and elastic, suggests that the tumour may have grown slowly to give the fibro-connective tissue time to proliferate and in turn to further check the rapid growth of the parenchymatous tumour tissue. The band of tumour tissue which caused the stricture of intestine may have grown more slowly than the base of the ulcer.

The origin of the tumour appeared to be at the base of the ulcer. At the edge of the ulcer, there were seen many single layers of atypical columnar cells forming gland-like structures adjacent to normal Lieberkuhn glands and some of them were even found in the midst of normal glands, which suggests that some of the cells in the Lieberkuhn glands were undergoing transformation into cancerous cells. Moreover some broken down Lieberkuhn glands contained cells which appeared to be in an intermediate stage between typical and atypical cells. Some of the cells in the normal Lieberkuhn glands were slightly enlarged and more intensely stained than normal gland cells.

These atypical cells which were found throughout from mucosa to mesentery have shown the singular characteristic of pseudo-gland formation as they spread throughout the neighbourhood.

The following evidence based on observation seems to suggest that the tumour originated from the cells of Lieberkuhn gland.

1. The attempted glandular formation, which however crude, suggests an inherited tendency toward gland formation.

2. The impression obtained was that the tumour cells are invading the neighbouring tissues, and undermining the healthy mucosa apparently arisen from a given point in the mucous membrane and radiating from it.

3. The larger alveoli containing necrosed masses of cells at the centre are found more numerous in the proximity of the intestine, but numerous small alveoli or merely clumps of atypical cells were found in the distal areas away from the intestinal mucosa. These facts indicate the probability that the origin of the tumour began at the mucosa. All of these direct

and indirect evidences are in favour of the view that the origin of the tumour was in the glands of Lieberkuhn in the intestinal mucosa, and the invasion of the neighbouring tissue seemed to take place through lymph channels or by direct extension.

This case is one of the very typical adenocarcinoma of small intestine which is the commonest type of carcinoma found in this part of the intestine.

B. CARCINOID TUMOUR

I. GENERAL CONSIDERATION AND DEFINITION

Considerable attention has been drawn lately to so-called carcinoid tumour of intestine, which is a tumour of small nodular size, usually multiple, in the wall of the small intestine and appendix, whose microscopical picture resembles somewhat that of carcinoma, but is generally believed on morphological grounds and from the viewpoint of malignancy, to differ from true carcinoma in being benign. The first report on this tumour was made by Lubarsch, who, in 1888, reported two cases, one with nodules and another with two nodules found scattered throughout the lower portion of the ileum. Lubarsch after studying these cases, reported them as primary carcinoma of ileum notwithstanding the fact that he was convinced of the difference between them and ordinary adenocarcinoma, because of a primary dissimilarity of cells, lack of metastasis, and absence of glandular structure, yet he traced them back to glands of Lieberkuhn of the intestinal mucosa. The term spheroidal cell carcinoma was used, but, nevertheless, he was inclined to believe them possibly originated from endothelial cells in the intestinal wall. The multiplicity of the nodules seems to be usual. Beside the two cases of Lubarsch, Bunting reported a case of six nodules and Nothnagel and Obendorfer both reported a case of three nodules. However this does not seem to be true in every case and there are a few reports of single nodules. The writer's present cases are of single nodules in both instances, one in jejunum, the other in ileum. All cases reported on appendix were single without any involvement in other parts of the intestine. In regard to the size of the nodule, no precise measurement of the tumour has been recorded in the literature. However, the tumour seems to be usually very small.

The site of the tumour is varied from jejunum to the upper part of the colon, but the most frequent site seems to be the appendix or ileum. Metastasis of this tumour has not been observed except in one case reported by Hansom in 1890, who observed metastasis in the liver of the individual. No relation of tumour to age and sex has been proved. They occur equally in both sexes, throughout life. A case as early as 5 years old was

reported by MacCarthy, and on the other hand, a case as late as 80 years old was reported lately by Primrose. However the tumour seems more frequent in middle aged persons than in the young or elderly individuals.

The microscopical pictures of this tumour described by many authors are identical in essential characteristics. A representative observation on the tumour is as follows:— The impression obtained on a first glance at the tumour is its resemblance to adenocarcinoma yet a careful study reveals a distinct dissimilarity from ordinary adenocarcinoma. The cells are usually present with oval and vesicular nuclei which stain poorly. The cytoplasm, also, is pale staining in appearance and minute vacuoles are contained within the cells. The formation of glandular tissue has been observed with cuboidal or columnar cells around a duct in single rows or more often clumps of cells forming alveoli surrounded by comparatively well stained cuboidal cells at the periphery and more irregular polygonal and vacuolated degenerated masses of cells in the centre of the alveoli. The alveoli of the tumour cells are surrounded by a fibrous stroma containing small amounts of muscular tissue, probably derived from muscularis mucosa. The epithelium of the mucosa is usually lacking and outwardly distended into the lumen of the intestine by expansion of the tumour, which occupies an enormously increased submucosa. In many cases including the first report made by Lubarsch and later by Nothangel, Walter and others, it is claimed that they have traced them to the glands of the mucosa, while others including Obendorfer stated that they found no connection with the mucosal glands.

The tumours are found as a rule at autopsy in small intestine or in appendix after appendectomy. All the cases reported of this tumour were those who had died of quite another disease, and had no symptoms whatever, referable to this tumour during their life time. Lubarsch's first case was found in a patient who died of tuberculous ulceration of the intestine, and another by Nothangel was in a patient who died of pneumonia, and Obendorfer's case was in a woman 30 years of age who died from typhoid fever. The writers two cases were one of lobar pneumonia, and the other of chronic nephritis.

In regard to the origin of this tumour there has been considerable controversy since the first report made by Lubarsch in 1888. It has been presented in literature as "endothelial carcinoma," "spherical celled adenocarcinoma" or "carcinoid tumour," and also as "primary carcinoma of appendix." Many divergent views have been advanced by various authors. A description of the various views in connection with the histogenesis of this tumour has been well outlined in short chronological order by Forbus in a recent issue of the Bulletin of Johns Hopkins Hospital.

In order to summarize the various views and theories advanced by different authors, they may be grouped under the following headings :—

1. *The Theory of Epithelial Cell Origin.* Lubarsch, who reported the first case of tumour of this type succeeded in tracing the tumour cells to the glands of Lieberkuhn was supported by many authors in their reports. Though Lubarsch finally reported his two cases under the title of " primary carcinoma of ileum " he was convinced that there were considerable differences between this and ordinary adenocarcinoma, and he suggested that the tumour may have originated from endothelial cells in the intestinal wall, after emphasizing a possible relationship of this tumour with certain inflammatory process observed in the intestine.

The epithelial origin theory was especially and firmly advocated by Hansom in 1890, who reported a case similar to those of Lubarsch and moreover he discovered wide metastasis of tumour in the mesenteric glands and mesentery and also multiple secondary growths in the liver. In 1896 Notthaft supported this view after obtaining evidence of metastasis in the neighbouring lymph glands. The epithelial cell origin had been advocated by many of the early workers but no reason was advanced for the marked difference from ordinary adenocarcinoma, and why they are benign while the adenocarcinoma are so malignant.

2. *The Theory of Endothelial Origin.* Although the possibility of endothelial cell origin of the tumour was advanced by Lubarsch, he nevertheless reported it as primary carcinoma. The first to put forward the theory of endothelial cell origin, was Glazebrook in 1895. He found a tumour in the appendix of considerable size, and after careful study noted its similarity to that reported by Lubarsch, and advanced his view of the endothelial origin of this tumour on the ground of its differences from adenocarcinoma and morphological resemblance of the cells to endothelial cells, and on the further evidence he secured as to the peculier relation of the cells to the greatly dilated lymph spaces and blood vessels. He referred to the tumour as " endothelial sarcoma." The example was followed by Obendorfer and Kelly as " lymphatic endothelial carcinoma " as they failed to trace them to gland in mucosa and the coexistence of this tumour with tuberculosis in the same organ seems to have Played a part in confirmation of a diagnosis of tumour of endothelial origin.

3. *Basal Cell Origin Theory.* This view of carcinoid tumour was suggested by Bunting in 1904, on the grounds of the marked morphological resemblance of this tumour to basal cell carcinoma of dermal epithelium first described by Krompecher, and later in 1919 Krompecher, after a careful study of this tumour approved the theory advanced by Bunting and came to the conclusion that the carcinoid tumour of the intestine is

analogous to the basal cell carcinoma of the skin and they may occur as solid cylinders, or adenoid masses. Bunting's view was supported by Buckhard in 1909 in his careful study of tumour.

4. *Pancreatic Rest Origin Theory.* The malformation of intestine due to an embryonic rest of pancreatic tissue on the intestinal wall, has frequently been discovered, especially in duodenum, jejunum where it has been described as "implanted accessory pancreatic tissue in intesttne." This is very often found on the top of Meckel's diverticulum. The theory relating this pancreatic rest as a possible origin of carcinoid tumour was advocated by Trappe in 1906. According to the opinion of Trappe, the adenomyoma and the carcinoid tumours are identical but vary slightly in the degree of their development, both originating from pancreatic rest in intestine. This embryonic tissue is found either in the submucosa or in the muscular coat of the intestinal wall. The adenomyoma is a tumour which is a highly developed outgrowth of this rest and consists of well differentiated glandular tissue with well formed ducts which are found to communicate with crypts of Lieberkuhn and on the other hand the carcinoid type is of very low order of development in which the epithelial cells have undergone no differentiation but remained in a disorganised state, however with an usual capacity for growth. This view was advocated by Obendorfer and others, and was based merely on morphological investigation. Later Taenniessen 1910 and Saltykow modified the original view of Trappe and preferred to regard the tumour as arising not from pancreatic accessory tissue in small intestine, but from partial misplacement of pancreatic tissue in which only the islands of Langerhans were represented, basing this upon the striking resemblance of the carcinoid tumour to the cells of the islands of Langerhans, but still admitting the common origin of the carcinoid and adenomyoma. Among many authors who agreed with this view were Schmidt and Obendorfer. Schmidt admitted the theory of pancreatic rest origin but doubted its pure islet character in the carcinoid type and Obendorfer called attention to the fact that the protoplasma of carcinoid cells give striking chromaffin reactions and contain abundant doubly refractible substances within the cells, but still emphasized the Taenniessen view of pancreatic rest origin on morphological and embryological grounds.

5. *Argentaffin Cell Theory.* It is a well known fact that the epithelium and glands of the intestine contain among its columnar cells certain cells which react to chrome salts when the tissue is fixed in Zenker's fluid and to which a name of Schmidt cell was given after the discovery of the cells by Schmidt in 1905, he also observed that these chromaffin cells have an affinity for silver. The relation of this Schmidt cell with

Klutsky cell which was described in 1897 is still obscure, but they are generally believed to be similar cells. It was suggested by Hubschmann in 1910, because of the yellow gross appearance of the tumour nudule, that the carcinoid may have their origin in this "Gelben Zell" of the intestinal mucosa, which also have a chromaffin nature. Gosset and Masson who had made an extensive study of these cells in the normal mucosa of the intestinal tract, proved that these chromaffin and also argentaffin cells were found throughout the intestinal mucosa and suggested that they be placed in the same category as cells found in the islands of Langerhans, and may have a similar function in endocrine secretion. This chromaffin and argentaffin cell origin for carcinoid was advanced by Hubschmann, Gosset and Masson in 1914, who stated that the large quantity of lipoids contained in carcinoid cells is an essentially important point in the differentiation of this tumour from adenocarcinoma.

This seems to be a very modern view of the origin of carcinoid tumour and a few reports in recent years, especially that of Hasegawa in 1923 and Danisch in 1924 confirmed this theory.

The relationship of carcinoid tumour with tuberculosis was suggested by many authors on the ground of its frequent association with tuberculosis, and the fact that carcinoid tumours are more frequently found in the appendix and lower part of the ileum, where tuberculosis is most frequent too, and also on the superficial resemblance of the nodular form of tuberculosis to the carcinoid tumour. The tubercular nodules in this region of intestine are found to be hyperplastic in nature instead of the destructive character usually found elsewhere. Milner after investigating 14 cases of primary carcinoma of appendix (carcinoids) remarked upon the striking resemblance of the two tissues, and he suggested that the carcinoids are the product of a chronic hyperplastic inflammation, chiefly a hyperplastic lymphangitis and the cells of endothelial instead of epithelial origin. Lubarsch's case was found in a patient who died of a tuberculous intestinal ulcer, Hinz reported a case of carcinoid tumour accompanied by tuberculosis, and there are many others who found tuberculosis in some other region of the body when carcinoids were discovered. Primrose in reporting a case a carcinoid tumour of the ileum, after through study of the literature, stated that the simultaneous existence of tuberculosis and carcinoid appeared to be more than mere coincidence and asserted that the tuberculosis may in many instances possibly play an important part in production of carcinoid tumour. The writers cases had obsolete pulmonary tuberculosis.

In tuberculosis, the nodule may present a tissue element which consists of more fibro-connective tissue and a comparatively small number of

endothelial cells with not infrequent giant cells. On careful comparative study of these conditions though they resemble each other, especially in the appendix, the difference may be noted between them as to general structure of nodules, or in individual cells which are believed to be more allied with the epithelial type of cells rather than the endothelial cells. Glandular formation has been observed in all cases of carcinoid tumour, the smaller the alveoli the more distinct in appearance. Recent studies of this tumour based, not only on morphological and embryological grounds, but also on microchemical methods of investigation, suggest some possible connection of this tumour with tuberculosis and subsequently support the view of endothelial origin, do not seem to take hold of any ground except as a matter of historical interest. However there seems to be some connection with certain types of inflammation to carcinoid tumour. How and in what manner the inflammation should influence the formation of epithelial tumour cannot be told. But based on observations of the cases, especially on another case in the appendix which will be stated later, the inflammation seems to play a part in production of carcinoid tumour, especially in the appendix.

II. THE MATERIALS, THE METHOD AND THE AIM OF THE PRESENT STUDY

The materials consist of three carcinoid tumours, one in jejunum, one in ileum and one in appendix; that found in jejunum was previously reported by Dr. A. Primrose of the Toronto General Hospital.

In the morphological study the ordinary routine methods which consisted of formalin fixation and haematoxylin eosin staining were used, and for study of the chromaffin granules, Zenker's fixation followed by haematoxylin eosin stain and also a special Giemsa's stain for tissue was applied at the suggestion of Professor Robinson. In studying the lipoid contents of the cells, frozen sections were stained with Scarlet red, and Van Gieson's stain was used for study of connective tissue of stroma.

The argentaffine nature of the granules of the carcinoid cells, was studied by staining the block of tissue in 2 per cent silver nitrate solution according to the technique described by Hasegawa.

The present aim of study and the steps followed thereupon, can be illustrated in three headings:—

I To follow up the study of previous authors recorded up to the date on morphological and microchemical basis.

II Tracing the tumour cells, back to normal histology of intestine and to other organs.

III Comparison of carcinoid tumour with adenocarcinoma, basal cell carci-

noma of epithelium, hypernephroma, xanthoma for detection of the chromaffin and argentaffin quality of the cells.

Case I

Autopsy No. 82, 1927, Toronto General Hospital.

Patient aged 54, male, died from lobar pneumonia.

The history reveals little except some history of chronic alcoholism, persistent dry cough for seven years before present illness, and no evidence of intestinal disturbance. The finding of the tumour in the intestine was accidental. On examination of the intestine, a small hard nodule measuring 40. c m. in diameter was found beneath the mucosa in the middle portion of the ileum. This nodule was freely movable between the mucosa and the underlying coats of the intestine. The cut surface of the noduls was pale yellow in colour and glistening in appearance.

Case II

Autopsy No. 183, 1925, Toronto General Hospital.

The patient was a male, aged 80, died of chronic nephritis with haemiplegia.

The history was negative in regard to intestinal symptoms. The intestine showed a number of small cyst-like structures measuring 0.1 to 0.2 c.m. which lay beneath the mucosa and moved freely on the muscular layer. They were oblong in shape, lying transversely in the bowel. In the lower portion of the jejunum there was a large nodule measuring 0.5 c.m. in diameter, which was not movable on the muscular layer.

Case III

A carcinoid tumour of appendix was sent over from the maternity hospital which was found during a Caesarean section. No history was available in this case, various staining reactions were attempted on this material as it was fresh. The appendix was considerably swollen containing yellowish brown material which replaced the whole structure including the muscular coat. This measured 0.6 c.m. in diameter.

III. MICROSCOPICAL OBSERVATION

The microscopical observation of all these three cases was essentially similar. One is reminded of adenocarcinoma by the invasion into the neighbouring tissue, the false glandular formation, and by the formation of alveoli surrounded by stroma. However a striking difference from adenocarcinoma was observed in the size of the cells which were considerably smaller than ordinary carcinoma cells, with rather uniformly spherical or slightly oval nuclei well packed together in alveoli of varying size and shape forming a striking contrast to the large irregularly shaped cells found in adenocarcinoma. The seat of the tumour was in the wall of the

intestine between the mucosa and the muscular coat. A few remaining glands of Lieberkuhn were observed. The muscularis mucosa was also distended forming a sort of capsular band for the main portion of the tumour beneath it. The other end of the tumour was composed of bands of normal muscular caots and appeared to be somewhat thickened but contracted. The lateral boundaries were made up of fibrous bands which appeared to be supporting connective tissue of the adjacent rugae. The tumour occupies a large area forming irregular masses of cell nests, with alveoli of varying sizes and shapes. The smallest of the cell nests are composed of a few cells and the larger ones form definite ducts lined by columnar or cuboidal cells and the largest alveoli are seen as masses of packed cells with very poorly stained nuclei and cytoplasm but surrounded at the periphery by single layers of cuboidal cells, which stood out very prominently from the mass of cells within this layer, by reason of their deeply stained cytoplasm. The parenchymatous cell masses are surrounded by a stroma of fibro-connective tissue running in various directions circumscribing the tumour cell mass in the alveoli of varying sizes and shapes.

The alveoli are composed of cells which are polyhedral in shape and are fused together in conglomerate masses. The cytoplasm is pinkish grey in appearance and contains minute vacuoles. These are mostly clumps of cells in process of necrosis with pyknosed fragments of nuclei. The cells forming acini about the duct, within the alveoli or those at the periphery of the large alveoli, are columnar and cuboidal in shape, and their cytoplasm was seen to be rather deeply stained with eosin. The nuclei of the cells are generally sharply stained with delicate nuclear membrane and a few irregular chromatin dots within the membrane which give the nuclei a spherical or oval appearance. The nuclei in the columnar cells are seen in the outer part of the cell, leaving greater area of clear cytoplasm toward the lumen. The stroma was mixed with a small amount of muscular fibres which can be traced to muscularis mucosa where the muscle fibres are seen migrating into the tumour stroma. The stroma does not stain distinctly with eosin and appeared to be in a state of hyaline degeneration. The blood vessels were not numerous in the stroma but those found around the tumour showed a moderate amount of dilatation and were filled with red blood cells. Although a few lymphocytes were seen in the stroma together with a few eosinophiles, there was no sign of active inflammation in any part of the tumour. Many of the lymph channels around the tumour were dilated and small masses of tumour cells were seen in the lymphatics. The mucosa lacked villi and the epithelium retained only a few normal Lieberkuhn glands. The cells of the Lieberkuhn glands are

seen in most cases to contain large mucous droplets, and at the base of the acini are seen numerous paneth cells containing acidophilic granules within their cytoplasm. On the other hand the acini of canceroid cells are seen to have more sharply stained nuclei which are smaller and more spherical and containing no mucous droplets within their cytoplasm. A few of these acini are seen fused into an alveolar form, a few retaining the lumen of former acini, others entirely losing their former identity.

The muscularis mucosa is well retained and many branches of fibres were seen running downward and fusing with the stroma. The largest alveoli are seen immediately below the muscularis mucosa in the middle portion and also in the neighbourhood of the muscular coat. Smaller alveoli are scattered throughout and they are especially numerous near the muscular coat and at the periphery of the tumour. The numerous larger alveoli at the periphery of the muscular coat are considered to be comparatively old masses of tumour cells, and as a result of sudden cessation of the invasion of tumour cells by the thick muscular coat of the intestinal wall, have resulted in the formation of large alveolar masses. But innumerable small masses of cells around both sides of these alveoli indicate a very recent invasion which has not yet reached any considerable size, and the same explanation can be made for these small cell masses at the periphery.

The moderately sized alveoli are seen in fair numbers beneath the muscularis mucosa with the link of connective tissue leading up to those in the mucosa in a few places. Around the moderately sized alveoli immediately below the muscularis mucosa, numerous small patches of cells are seen, many of which have not yet formed glands but are spreading laterally on both sides undermining the normal mucosa immediately above.

All this evidence indicates that the tumour originated at the mucosa, most likely from the glands of Lieberkuhn and spread radially to the distal edges. If the tumour had started from the distal edges to the mucosa, there seems to be no reason for young cell nests concentrating at a particular point in the mucous membrane and also undermining the surrounding healthy mucosa, and transforming a few Lieberkuhn glands to tumour glands. Therefore, it seems safe to assume that the tumour originated from the glands of Lieberkuhn in the path of the mucosa to which the tumour has been traced. Further, since the lymph flow is outward from the mucosa, the invasion of tumour cells can easily take place in this direction. Moreover, many fairly regular glandular tissues have been observed in the mucosa in company with Lieberkuhn gland, which seems to justify one in presuming that the tumour cells could have originated from the glands of Lieberkuhn.

The next question is the identification of cells which are considered to have a common origin in the crypts of Lieberkuhn. Whether these two neoplasms originate in identical cells lining the Lieberkuhn glands and appear in different forms according to the circumstances under which they are exposed to certain unknown factors or originate from different distinct types of cells lining the crypts of Lieberkuhn which can be identified by the microchemical reaction of cells, if not by morphological investigations, is the reason for taking up the present work.

The morphological difference between these two tumours has been observed since the first case studied by Lubarsch. Bunting's study swung the pendulum to a basal cell origin. However the origin of the lipoid nature of the material contained in the carcinoid tumours has been a matter of dispute. It was not until the study of the tumour advanced from pure morphological grounds to microchemical investigation that the chromaffin and argentaffin nature of the granules contained in the sytoplasm of carcinoid cell was brought forward, and it was found that similar cells are said to have been found in normal mucosa and other parts of the body including the adrenal gland.

IV. THE STUDY ON CHROMAFFIN GRANULES

To study the chromaffin granules of the tumour cells, the tissue was fixed in Zenker's fluid for 24 hours and later stained either by haematoxylin eosin, or in special tissue stain of Giemsa which was suggested by Professor Robinson and has greatly facilitated the work. The tissue stained by this method presented a beautiful picture, colouring the muscular tissue in blue, the fibrous connective tissue in faint pink, red cells and eosinophile granules stained a bright red and all the nuclei a deep azure. The granules contained in the tumour cells stood out very conspicuously. The columnar and cuboidal cells which composed the outer layer of the alveoli and the same cells in the small alveoli forming gland acini, all contained dark brownish granules at the outer end of the cells, between the basal membrane and the nuclei which were situated at the outer third of the elongated cells. The granules were either obscure or altogether absent in the mass of polyhedral cells which were in the larger alveoli. The chromaffin granules are restricted to an area between the nuclei and the basal membrane, while no granules are observed in the cytoplasm proximal to the lumen of the acini which constitute two thirds of the cells. This area however, contained numerous minute vacuoles which appeared to be lipoid in nature. The boundaries of these pigment contained cells are clear, and are indicated by lines of pinkish staining, but the mass of cells within are not distinct in cell boundaries and the whole thing

appeared to be amorphorous except in having numerous pale staining nuclei within the mass. The pigments are arranged in such a way that when looked at as individual alveoli, they appeared to have a golden hoop around each alveoli. In the larger alveoli of the tumour tissue, the cell nuclei are of faint blue and the granules also very indistinct but still a trace of brown hoops are recognizable around the periphery. The smaller the alveoli which corresponds to younger cells, the more distinct are their shape and the cell inclusions; the brown pigmented granules and the lipoid substances, and on the other hand the larger alveoli, which seems to correspond to older cells, the more obscure or lacking in pigmented granules are they, but they still contained more lipoid material,

The fact that the cells contain chromaffin granules on one side near the base of the cells, and abundant lipoid material on the other side at the free edge and also that what appeared to be degenerated cells having lost their chromaffin granules, but are loaded with lipoid material, all seems to suggest that the lipoid substances within the cells are the results of the normal production of chromaffin granules and when the cells lacked nutrition as in the case of clumps of cell masses within the larger alveoli, the store of chromaffin granules was exhausted in formation of the lipoid substances and was not restored.

V. THE STUDY OF ARGENTAFFIN GRANULES

The argentaffine nature of chromaffin cells had been studied at an early date by Schmidt, and an application of this stain on carcinoid tumour was made by Gosset and Masson in 1914 and lately by Hasegawa.

A block of tissue was fixed in formalin solution for more than a few hours, and stained 24 hours in 2 per cent silver nitrate solution, kept in a dark bottle and incubated. After this another 24 hour period was passed in the incubator with the addition of 7 drops of ammonia to each 10 c.c. of the silver nitrate solution according to the method described by Hasegawa. A thin section was made from the block and examined. In the section of carcinoids of appendix thus treated, there were found to be three distinct types of alveoli scattered throughout each section. At the periphery near the muscular coat are seen numerous small clumps of dark granules in the fibrous stroma where no individual cells could be identified. These seem to me to be new alveoli, full of young cells loaded with chromaffin granules. At the proximal side near the centre of the appendix where the alveoli are of considerable size, no trace of black granules was observed, but the alveoli are composed of uniformly brown stained cells, the nucleus and cytoplasm staining alike. Between these two extremes, there are seen moderate sized alveoli, mostly composed of either a single

layer of columnar cells or a mass of polygonal cells within a single file of columnar cells. The cells stained slightly darker than those of the larger alveoli and a band of black granules were observed at the outer margin of each alveolus and under higher magnification these black granules are found in the protoplasm of the columnar cells between the nucleus and the basal membrane, or the exact position of brown pigmented granules that were seen in Zenker fixed tissues. From the study of these three zones of tissue with different staining qualities, one is impressed by the fact that the younger the cells the richer the argentaffin reaction of the cells, and the older the cells in the mass of the larger alveoli, the more this quality has been lost, all of which corresponds to the similar fact observed in chromaffin reaction.

With a view to study the lipoid nature of the carcinoid tumour, frozen sections were made and stained in Scarlet Red. In this section the alveoli are all filled with amorphorous red staining granules and neither cell boundaries nor nuclei could be recognized. However in the small alveoli, where a single lining of columnar cells is forming acini, a distinct picture was made out. The lipoid granules which stained red were seen in the proximity to the lumen of the acini and none were seen near the basal membrane. This lipoid material which seems to be normal product of chromaffin may be considered of possible endocrine nature as suggested by Gosset and Masson and others. Therefore the picture presented was that of a red band around the lumen of the acini, which indicates that the products of the chromaffin granules are lipoid in nature while the chromaffin granules do not stain with Scarlet Red. The amorphorous red granules which fill all the larger alveoli suggest that these are overloaded with lipoid material, partially due to normal product of chromaffin granules contained within the cells, and partially due to rapid degeneration of cells (fatty degeneration).

VI. STUDY OF NORMAL HISTOLOGY OF INTESTINE AND OTHER ORGANS

In order to permit the study of the cells in normal intestine which may give rise to carcinoid tumours, the similar chromaffin and argentaffin stains were applied to the tissues taken from stomach, duodenum, ileum, appendix and colon, and beside these materials some of the other organs such as pancreas and adrenal have also been studied in the same way. Lastly a comparative study of cells was made on basal cell epithelioma, xanthoma and hypernephroma on both chromaffin and argentaffine sections. Throughout the gastro-intestinal tract are seen the Schmidt cells with their granules stained a brownish yellow or dark brown to black in silver nitrate

stain. These cells appeared to be very delicate in nature and prone to undergo an early degeneration of granules. The tissue obtained at autopsy as early as three hours after the death, did not show the granules distinctly and therefore it was necessary to secure the material for study entirely from surgical specimens.

On examination of normal tissues of stomach and intestine both small and large, the chromaffin and argentaffin cells were observed throughout the gastro-intestinal tract. They are seen among normal epithelial cells in villi and also among the lining cells of the crypts of Lieberkuhn. They seem to be more numerous in the appendix more especially in the glands rather than the exposed epithelium. These cells were most numerous in the appendix and ileum and they are rather sparsely distributed in the stomach and colon. This fact seems to coincide with the more frequent occurrence of carcinoid in these regions of the intestine. In general appearance the cells of this type are similar to the rest of the epithelial cells. The nuclei of the cells are seen pushed forward from the file of the rest of the nuclei of the cells by the pressure exerted by the granules which fill the space of the cytoplasm between the nucleus and the basal membrane. The nuclei of the cells are generally more or less spherical rather than elongated which is the shape of the ordinary epithelial cells, and stain a palor colour than the rest. In some places these cells are compressed between the mucous cells and assume an elongated shape but they appeared to be smaller and delicate in appearance. Black granules or brownish yellow granules are always visible at the basal side of these cells. The general appearance of the nuclei resembles that of the basal cell which are found located deeply in the basal part of the gland and are often triangular in shape with narrow end in the lumen and the broad base at the basal membrane. The nuclei are always faintly stained and the nuclear membrane has a fine beaded appearance. Numerous basal cells have been seen in normal condition within the glands of Lieberkuhn, and the suspected origin of carcinoid tumour from these cells cannot be proved.

For a comparison adenocarcinoma of stomach, jejunum and colon were stained both by chromaffin and argentaffin stains but so far no positive records have been obtained. Because of the morphological similarity of the cells of carcinoid tumour with basal cells of normal intestine and these in the islands of Langerhans in the pancreas and also those in the medulla of the adrenal described by previous authors, a careful study was made of these organs. Since the study was made from autopsy material, the result seems rather unreliable if our experience with the gastro-intestinal mucosa obtained from autopsy material did not show the characteristics of the looked-for cells even three hours after death. However, it seems to

be a general fact that the islands of Langerhans contain cells which on morphological study resemble to those found in normal mucosa or in carcinoid tumour and on microchemical investigation these cells react very feebly to both silver nitrate stain and chromaffin stain. In adrenal, the result so far as silver stains is concerned, is found to be negative although a slight tinge of chromaffin granules was observed in the cell of the medulla. Since the chromaffin stain varies widely in extent even in normal medulla of adrenal in different individuals at different ages, no definite emphasis can be put on this result. A morphological resemblance of the cells of carcinoid tumours with those basal cell epithelioma led to the study of the latter tissue with both chromaffin and argentaffin stains which resulted in a failure to demonstrate any chromaffin or argentaffin granules possessed by these cells. The morphological study also revealed that there was no attempt on the part of the tumour to form glands within the alveoli. It was in tumours whose alveoli were filled with small polygonal cells surrounded by a single layer of cuboidal cells at the periphery that no lipoidal formation was demonstrable in the cells as in the case of the carcinoid tumours. These facts show that there is no connection between the carcinoid tumour and basal cell epithelioma.

The yellowness and fatty character of certain tumours, the hypernephroma, and xanthoma and lutein cyst of ovary led to their being stained in silver nitrate solution, but all failed to demonstrate fatty contents of the cells by this stain except as white vacuoles.

C. SUMMARY AND CONCLUSION

1. Carcinoma of intestine can be placed in two categories; true carcinoma and the carcinoid tumours which are, in reality, two distinct types of tumour.

2. In intestine the adenocarcinoma of constricting (ring formation) type is the most common form of carcinoma, although the papillary form is said to be common in the first part of the duodenum. In the appendix primary carcinoma are mostly of the carcinoid type.

3. The adenocarcinoma originate in the crypts of Lieberkuhn from ordinary epithelial cells, while the carcinoid tumours, though also originating in the crypts of Lieberkuhn, develop from the cells of the same epithelium, but contain particular granules, whose presence can only be demonstrated either by staining with silver nitrate solution, or by ordinary haematoxylin eosin stain after fixation in Zenker's fluid. The best result, however, was obtained from the special Giemsa stain.

4. In studies of the normal histology of the gastro-intestinal tract these argentaffin and chromaffin cells were found distributed throughout

the mucosa of the stomach, small intestine, appendix and colon. They are more numerous in the glandular cells than in the exposed surface of the mucosa, and further my impression was that they are more numerous in the appendix and ileum than in the rest of the alimentary tract. This fact seems to coincide with frequency of the carcinoid tumour in these parts of the intestines.

5. Microscopical study reveals the fact that they are entirely different tumours, carcinoma has large atypical cells with nuclear which are irregular both in size and shape. The destruction of tissue was most marked as the tumour invaded the surrounding tissue, while on the other hand the carcinoid tumours possessed atypical but uniformly small cells whose nuclei are vesicular and almost regular in size and shape. No destruction of tissue was evident as the tumour invaded neighbouring tissue. Both were attempting gland formation within the alveoli and contain stroma of fibrous connective tissue but in carcinoids a considerable amount of muscular tissue as well was found in the stroma and in both cases the cells could be traced to the crypts of Lieberkuhn.

6. The microchemical reaction differs altogether in these two tumours. The carcinoid cells contained granules of chromaffin and argentaffin nature while no such granules are evident in adenocarcinoma.

7. Metastasis is rare in adenocarcinoma of small intestine and only few cases were found in the literature with metastasis to other parts and in the present case no metastasis to the mesenteric glands was found. In carcinoid tumours metastasis was reported only once and in the present three cases no metastasis was evident in any part of the body. But, in so far as the local invasion of tissue is concerned they are both to be considered malignant.

8. A comparative study of carcinoid tumours, basal cell epithelioma, hypernephroma, and xanthoma, was attempted and no possible connection of any nature was evident among them.

9. Study of the isles of Langerhans and the medulla of the adrenal showed some slight similarity in staining to the cells of carcinoid but no definite proof was obtained.

10. As to the histogenesis of carcinoid tumours, no proof was obtained of either an endothelial or basal cell origin. The theory of pancreatic rests cannot be substantiated in the light of the present study. The view recently advanced by Gosset and Masson originally suggested by Hubschmann that the carcinoid tumour has its origin in the chromaffin cell of the intestinal mucosa and may be termed as "endocrinal tumour" seems to fit in with the results of the present study.

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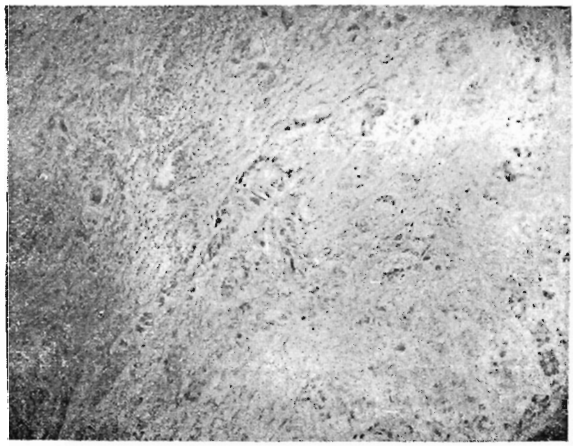
ADENOCARCINOMA

80×



CARCINOID TUMOR

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