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EXPERIMENTAL STUDIES ON THE INFLUENCE OF RETICULO-ENDOTHELIAL SYSTEM UPON THE INDUCTION OF MALIGNANT GASTRIC NEOPLASMS

by

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Received for publication May 25, 1963

INTRODUCTION

YAMAGIWA and ICHIKAWA (1915)^{64, 25)} were the first to succeed in producing skin cancer in rabbits. Liver cancer, pulmonary cancer, mammary cancer and other organic cancers were produced later relatively easily in animals. However, the production of gastric cancer identical in histogenetical points with human gastric cancer experimentally was difficult despite the efforts of many investigators. Experimental gastric cancer in rats which was reported for the first time by FIBIGER^{11, 22)} in 1913 was nothing more than a papillary growth of the squamous epithelium of the forestomach, and was found to be entirely different from gastric cancer in man.

STEWART, HARE, LORENZ, BENNETT, SNELL^{17, 20, 47, 48, 49, 50, 53,)}, FIRMINGER¹²⁾, COLLINS, GARDONER and STRONG¹⁰⁾, have succeeded in the production of precancerous lesion or adenocarcinoma in the glandular stomach of rats and mice by oral administration of carcinogenic polycyclic hydrocarbon, 20-methylcholanthrene, and by intramural injection of these substances into the submucosa of the glandular stomach. The incidence, however, was low.

STEWART et al.^{51, 29)} has recently reported to have succeeded in producing the above mentioned change in the glandular stomach of rats by means of oral administration of N-N'-2,7-fluorenylene bisacetamide at a fairly high rate.

The author¹²⁾ has obtained data by investigating various factors in producing malignant tumors of the stomach experimentally, by the injection of a suspension or emulsion of 20-methylcholanthrene into the submucosa of the glandular stomach of the rat, resorting either to the local simultaneous injection of ethanol or to the blocking of the reticulo-endothelial system (splenectomy, X-irradiation, injection of pigments, etc.).

MATERIALS AND METHODS

(I) Materials

i) Carcinogenic agent^{13, 14)} : As a strong carcinogenic polycyclic hydrocarbon, 20-methylcholanthrene (MC) (Fluka Co., Switzerland) was used. In order to perform injection into the gastric wall of rat, MC-methocel suspension (4%) and MC-olive-oil emulsion

Table 1

(1) Composition of MC-Methocel Suspension :	
	Grams percent
20-Methylcholanthrene	4.0
Aerosol OT	0.01
Methylcellulose (4000)	0.2
Ethylalcohol	1.0
Distilled water - q. s.	100.0
pH adjusted to 7.4 with 0.1N NaOH	
(2) Composition of MC-Olive-Oil Emulsion :	
	Grams percent
20-Methylcholanthrene	4.0
Olive-Oil - q. s.	100.0

(4%) were prepared according to the method described by LORENZ, HARE and STEWART. For their composition, refer to Tab. 1²⁷⁾.

ii) Experimental animals : Wistar-strain rats obtained from the Animal Center Laboratory of the Kyoto University Medical School were used. A total of 404 male rats, about 80 to 100 gram of body weight and 4 to 5 months of age were used. They were caged in groups of 5 to 8 each,

and maintained on a diet of "Oriental laboratory chow" (Oriental Yeast Mfg. Ltd, Japan), tap water and vegetables.

(II) Experimental procedure

As presented in Tab. 2, the effective experimental animals numbered 404. The animals were divided in 2 groups, MC-methocel suspension group (M-group) and MC-olive-oil emulsion group (O-group) by use of MC-solvent. These groups were then subdivided in the following 6 groups. Using a sterile tuberculin syringe, injection was performed.

i) 0.01 cc of methocel suspension containing 0.4 mg of MC (Tab. 1) and 0.01 cc of 90% ethanol was injected into the pyloric submucosa of the anterior wall of the glandular stomach (M-I).

ii) 0.01 cc of olive-oil emulsion containing 0.4 mg of MC and 0.01 cc of 90% ethanol was injected into the pyloric submucosa of the anterior wall of the glandular stomach (O-I).

iii) 0.01 cc each of the MC-suspension was injected into the submucosa of the pylorus and the corpus of the glandular stomach (M-II).

iv) 0.1cc each of the MC-olive-oil emulsion was injected into the submucosa of the pylorus and the corpus of the glandular stomach (O-II).

v) 0.01 cc of the MC-suspension and 0.01 cc of ethanol was injected into the pyloric submucosa of the glandular stomach, performing splenectomy at the same time (M-III).

Table 2

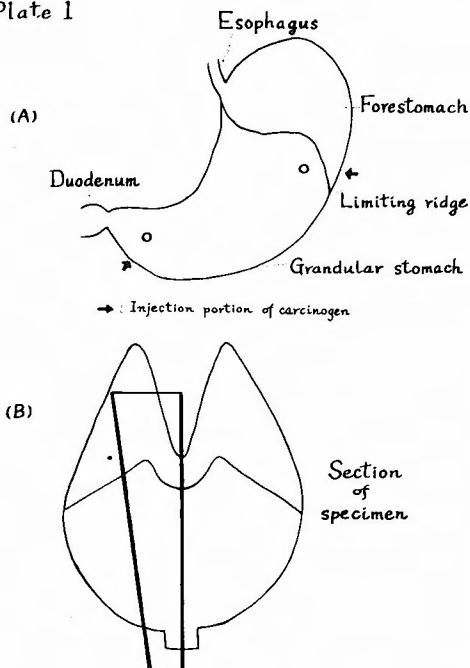
Intramural injection of carcinogen and other treatments	M-Group 20-Methylcholanthrene Methocel Suspension	O-Group 20-Methylcholanthrene Olive-Oil Emulsion	Totals
Number of rats	215	189	404
MC-4% 0.01cc (0.4mg) and Ethanol 90% 0.01cc (pyl.)	143	129	272
MC-4% 0.02cc (0.8mg) (pyl. corp.)	35	12	47
MC-4% 0.01cc (0.4mg) and Ethanol 90% 0.01cc (pyl) Splenectomy	37	48	85

vi) 0.01 cc of the MC-olive-oil emulsion and 0.01 cc of ethanol was injected into the pyloric submucosa of the glandular stomach, performing splenectomy at the same time (O-III).

The operative procedure was carried out under nembutal anesthesia which was administered intraperitoneally. The abdomen was shaved and the stomach was delivered through a midline incision in the epigastric region. By means of a 0.5 cc tuberculin syringe, a

suspension or emulsion of MC and ethanol was injected tangentially into the serosal surface of the anterior wall in the portion of the glandular stomach. The technical success of the injection was shown by the presence of a small yellow bleb of ca 2 mm in diameter at the site of the injection. The first injection-site was the wall of the antrum near the pylorus (pre-pyloric region), the second injection-site was the wall of the corpus (fundic region) of the glandular stomach at a point not less than 0.5 cm distal to the limiting ridge. The splenic artery and vein were ligated and the spleen was removed in the splenectomized group. Following the injection, the stomach was returned to the peritoneal cavity and the abdominal wound was closed by silk-thread suture.

Plate 1



RESULTS

One animal in each group was sacrificed every hour from the 1st to the 24th hour after treatment. From the 2nd day to the end of the 2nd week, one animal was sacrificed each day, thereafter, 3 to 5 animals were sacrificed every week, allowing observations for 607 days after the injection, at the longest. The animals which died of other causes than tumor were excluded from this studies. The animals were laparotomized immediately after sacrifice and were examined. The stomach was isolated together with the inferior part of the esophagus and a part of the duodenum and were fixed in 10% formalin solution. Paraffin sections were prepared from 2 to 4 areas of the anterior wall of the stomach, as presented in Plate 1. The sections were put to hematoxylin and eosin staining. In a number of sections, van Gieson staining and the silver impregnation method for reticulin fiber were employed.

(I) Macroscopic observations

i) Changes in the abdominal cavity : Bloody ascites retention was noted in 2 sarcomas (220 and 148 days after MC-injection, respectively) and in one adenocarcinoma

(303 days after injection) of the group given MC intramural injection at the pylorus and corpus of the glandular stomach, and also in 8 sarcomas (191, 170, 204, 214, 199, 212, 189 and 190 days after injection, respectively), and in 2 adenocarcinomas (168 and 149 days after injection, respectively) in the splenectomized group. In 4 instances showing liver metastasis in the splenectomized group, the great amount of bloody ascites retention was observed. In 10 cases of the malignant tumors developed by intramural injection of MC into the glandular stomach, macroscopic metastases in the other organs were observed. In the splenectomized group, adenocarcinoma was observed in 2 animals (168 and 149 days after MC-injection, respectively) and sarcoma in 8 animals (206, 186, 220, 148, 199, 212, 189 and 190 days after MC-injection, respectively) which produced liver metastasis.

Liver metastasis of sarcoma with relatively distinct delineation produced a hard tumor formation on the surface of the liver. But the liver metastasis of adenocarcinoma did not form as hard masses. Lymph node swelling could not be discovered in any of the animals with metastasis. Liver abscess was noted in 4 animals and liver cyst in 8 animals.

Adhesion was recognizable on the lapse of one to two weeks after the MC-injection. The marked adhesion generally developed on the lapse of 2 months after injection, and a most intense fibrous adhesion occurred between the MC-injected serous membrane of the gastric anterior wall and the liver surface or its bed. It also involved the spleen, omentum, duodenum, pancreas and peritoneum. In the splenectomized group, even shortly after the MC-injection, an intense and extensive adhesion was generally observed in many of the animals.

ii) Changes in the stomach : Macroscopic tumor formation and ulceration were noted following MC-injection alone in 101 of 319 animals (M-I, M-II, O-I and O-II) (31.8 %), and following MC-injection with splenectomy in 50 of 85 animals (M-III and O-III) (58.8%). The first ulcer was found at 59 days after the MC-injection. Of the malignant tumors, sarcoma was observed in 20 animals, which was palpable in all of them.

Giant ulceration was noted in 2 of 5 animals with adenocarcinoma, while in the remaining 3 animals, neither tumor nor ulcer was distinct in the serous membrane and mucous membrane. Mucosal erosion was observed in 26 animals, one of which belonged to the splenectomized group. No neoplastic change was observed macroscopically in the forestomach.

(II) Histological findings

Following injection of MC into the anterior wall of the glandular stomach in rats, round-cell infiltration and polynuclear leucocytic infiltration developed in the submucosal tissue at the site of injection. This was followed by ulceration, and then by adenomatous proliferation and/or adenocarcinoma formation. In some animals, sarcoma was noted to develop. The average life-span in the rat is believed to be from 2.5 to 3 years, while animals in the present experiment survived to 607 days after the last MC-injection. These animals were divided into the following groups and the histological changes in course of time were examined (Tab. 3).

Group I received intramural injection of MC and ethanol at the pylorus (M-I and O-I).

Table 3

Intramural injection of carcinogen • Other treatments	20-Methylcholanthrene Methocel suspension				20-Methylcholanthrene Olive oil emulsion				Totals
	MC 4% 0.01cc (0.4mg) • Ethanol 90% 0.01cc (pyl.) (M-I)	MC 4% 0.02cc (0.8mg) (pyl. corp.) (M-II)	MC 4% 0.01cc (0.4mg) Ethanol 90% 0.01cc (pyl.) Splenectomy (M-III)		MC 4% 0.01cc (0.4mg) • Ethanol 90% 0.01cc (pyl.) (O-I)	MC 4% 0.02cc (0.8mg) (pyl. corp.) (O-II)	MC 4% 0.01cc (0.4mg) • Ethanol 90% 0.01cc (pyl.) Splenectomy (O-III)		
Number of rats	143	35	37	215	129	12	48	189	404
Adenocarcinoma (with liver metastasis)	2	0	0	2	1	0	2 (2)	3 (2)	5 (2)
Cancer-like invasive lesion	37	2	11	50	11	0	14	25	75
Sarcoma (with liver metastasis)	0	2	8 (4)	10 (4)	6	0	4 (4)	10 (4)	20 (8)
Ulceration	53	19	22	94	29	0	26	55	149
Adenomatous proliferation	95	24	32	151	53	4	31	88	239
Adenoma	2	2	1	5	3	0	1	4	9
Intestinal metaplasia	4	0	2	6	6	0	3	9	15
Squamous metaplasia	22	3	3	28	12	3	5	20	48

Group II received intramural injection of MC at the pylorus and corpus (M-II and O-II).

Group III received intramural injection of MC and ethanol at the pylorus and splenectomized (M-III and O-III).

1) Group of intramural injection of MC and ethanol at the pylorus, 272 animals (M-I and O-I) :

Results in this group were as follow,

*Adenocarcinoma	3 (1.1%)
Cancer-like invasive lesion	48 (17.6%)
Sarcoma	6 (2.2%)
Ulceration	82
Adenomatous proliferation and adenoma	153
Intestinal metaplasia	10
Squamous-cell metaplasia	34

(a) Adenocarcinoma was observed in 3 animals (223, 303 and 160 days after MC-injection, respectively).

*Animal 1 with adenocarcinoma autopsied at 223 days after the MC-injection (M-I) (Figs. 1, 2 and 3). In macroscopically, no tumor or ulcer was found in any part of the gastric tissue. In the microscopic examination, the mucous membrane of the glandular stomach was in marked hyperplasia and proliferation. A part of the connective tissue of the pyloric submucosa exhibited a high degree of round-cell and polynuclear leucocytic infiltration.

A proliferation pattern of cells indicating an atypical glandular structure was observed at this area. Cells constituting the atypical glandular structure had both nucleus and cytoplasm larger than normal and irregular in size. The nucleoplasm was coarse

Explanation of Microphotographs

Figure 1. Adenocarcinoma of the glandular stomach of a rat 223 days after injection of MC (M-I). There is remarkable proliferation of atypical glandular epithelium at the base of the pyloric ulcer. The neoplastic lesion is invaded into the subserosal tissue, in partially. Mucous membrane to the above. Hematoxylin and eosin. $\times 28$

Figure 2. A high-powered view of a portion of the atypical glands shown in figure 1. The cells composing this glandular structure are irregular in size and shape, and also showed atypical mitosis. Hematoxylin and eosin. $\times 100$

Figure 3. This is a higher power view of a portion of figure 1 and 2. Hematoxylin and eosin. $\times 400$

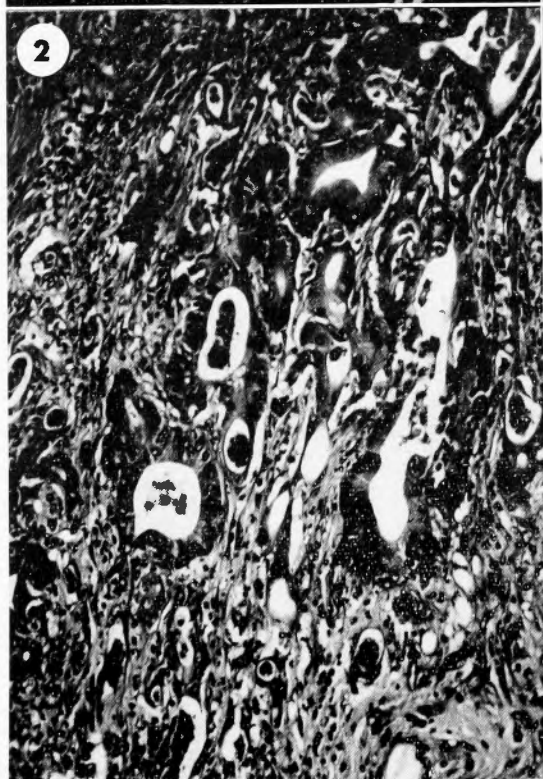
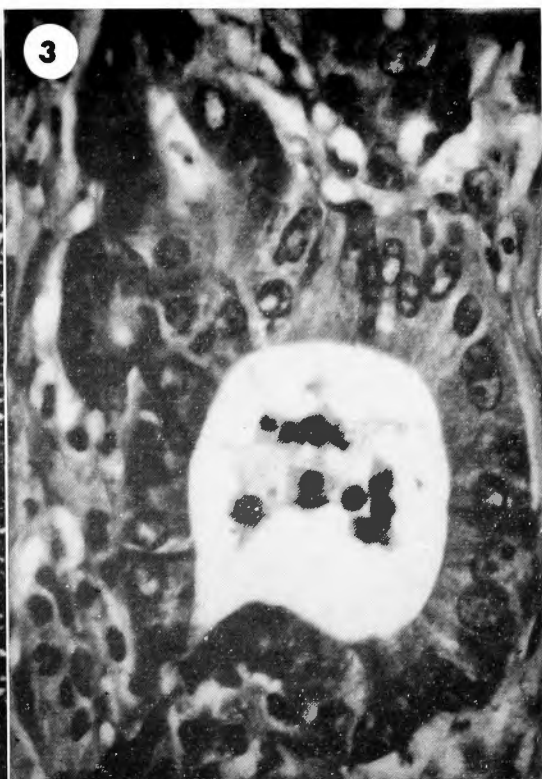
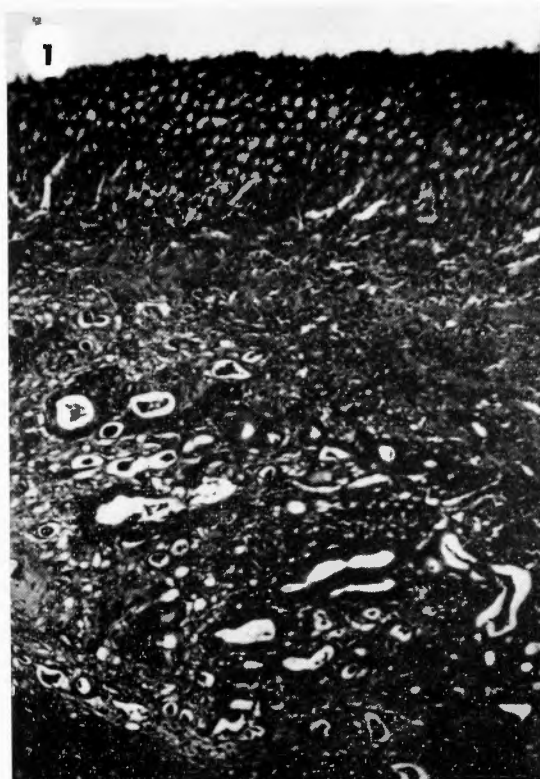
Figure 4. Adenocarcinoma of the glandular stomach of a rat 168 days after injection of MC and splenectomy (O-III). The neoplastic lesion has infiltrated into the subserosal tissue of the glandular stomach. Mucous membrane to the above. Hematoxylin and eosin. $\times 28$

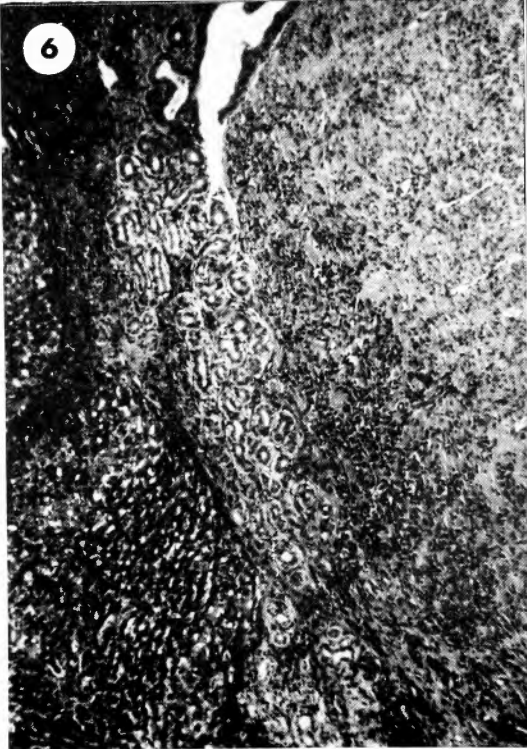
Figure 5. A high-powered view of an area of the atypical glands in figure 4. Proliferation of the atypical glands has extended through the muscularis mucosae into the submucosa. Hematoxylin and eosin. $\times 100$

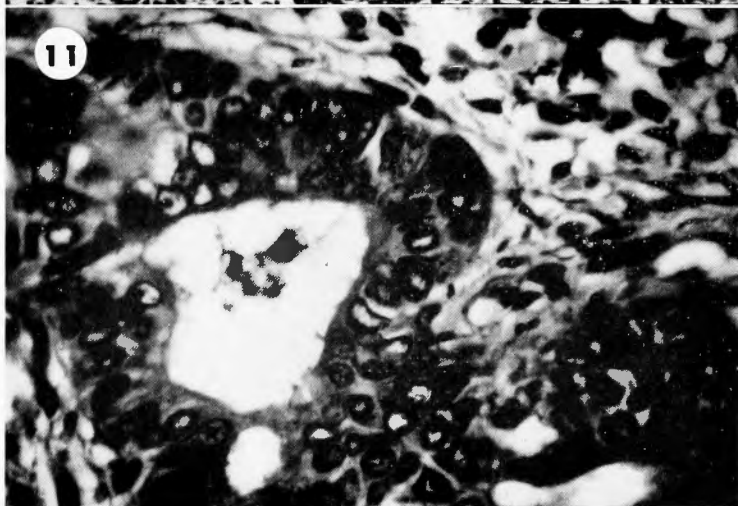
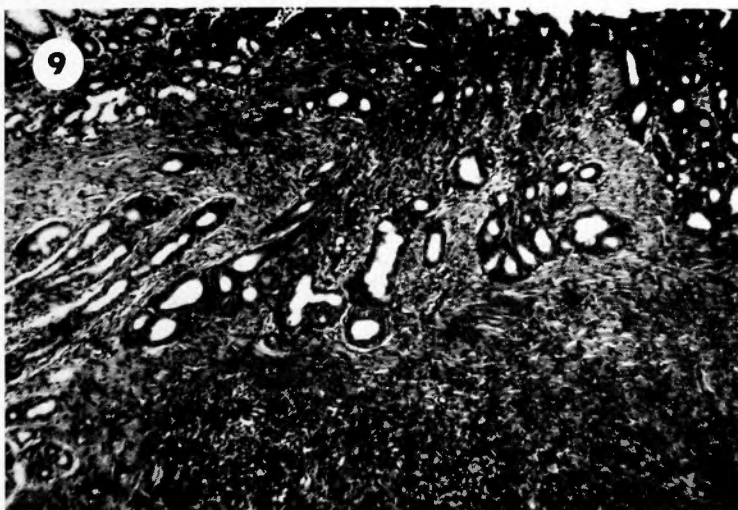
Figure 6. Adenocarcinoma of the glandular stomach with liver metastasis of a rat 149 days after injection of MC and splenectomy (O-III). Invasion of the atypical glandular epithelium with alveolar structure has extended through gastric mucosa, muscle layer and serosa into the intra-hepatic tissue. Round-cell infiltration can also be seen in this lesion. Liver tissue to the left. Hematoxylin and eosin. $\times 28$

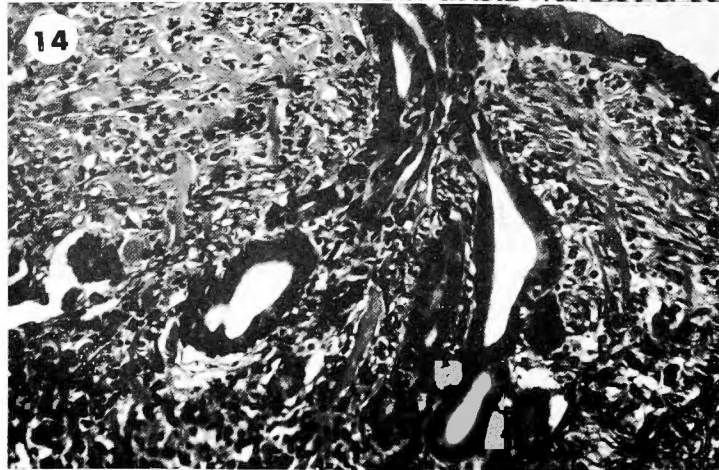
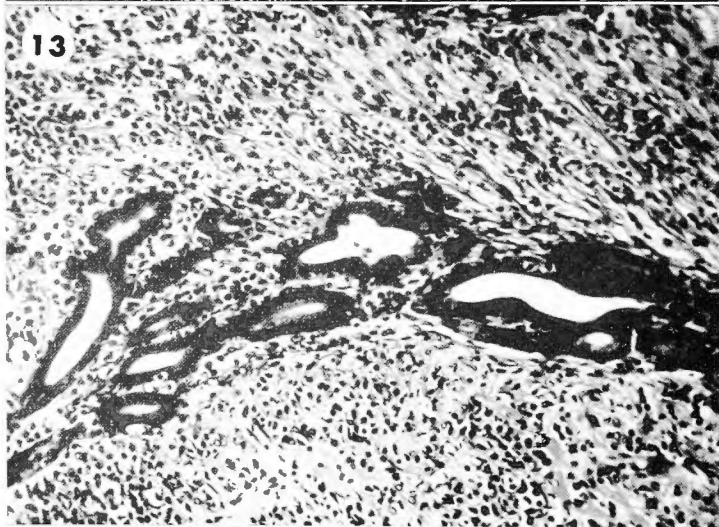
Figure 7. A high-powered view of an area of figure 6. Atypical glandular lesion occurring in the liver tissue. Hematoxylin and eosin. $\times 100$

Figure 8. This is a higher power view of a portion of the atypical glandular structure shown in figures 6 and 7. Hematoxylin and eosin. $\times 400$









and had one or two nucleoli, and the cells revealed an irregular arrangement.

*Animal 2 with adenocarcinoma autopsied at 303 days after the MC-injection (M-II). No particular lesions were found in the serosa of the glandular stomach and perigastric organs. There was also no evidence of tumor or ulcer formation at the mucous membrane of the glandular stomach. Presence of an adenocarcinoma in the muscle layer was observed microscopically. Atypical features of tissue were found including irregular size of the cells. Round-cell infiltration around the adenocarcinoma was evidenced.

*Animal 3 with adenocarcinoma autopsied at 160 days after the MC-injection (O-I). A wide and strong adhesion was present between the serosa of the anterior wall of the glandular stomach and the surface of the liver. A hard, grey and nodulated tumor was palpable on the serosa. An intense ulceration was noted on the mucous membrane of the pylorus, corpus and cardia. The base of the ulcer exhibited round-cell infiltration. The tumor was found to be adenocarcinoma spreading in the submucosal tissue histologically.

(b) Cancer-like invasive lesion was noted in 48 animals. The invasion occurred in the period from the 59th (Figs. 12 and 13) to the 598th day after the MC-injection. Fairly wide ulceration was generally present from the mucous membrane of the pylorus to the corpus. Adenomatous proliferation of cells were observed in some areas from the ulcer surface to the deeper layers. The adenoma cells were somewhat irregular in size. The nuclei were round, oval or irregular shape and had clear nucleolus and nucleoreticulum occasionally.

The ulcer base had a proliferation of granulation, fibrous and hyalinized connective tissues, and also firmly adhered to the liver. The granulation tissue had invaded deep into the hepatic lobules in an irregular manner, and was proliferating there. The liver-cell-trabecula was in very disordered arrangement, with liver-cell proliferation here and there. The glandular epithelial cells around the ulcer exhibited varying degrees of hyperplasia. The liver tissue indicated degenerative atrophy of cells. The lumen showed stenosis due to hyperplasia of the blood vessel intima.

The mucous membrane of the glandular stomach was thickened, especially at the

Figure 9. Cancer-like invasive lesion of the glandular stomach of a rat 87 days after injection of MC and splenectomy (M-III). There are remarkable proliferation of pyloric glandular cells with slight atypicality from the superficial layer of the ulcer into the submucosal connective tissue. At the upper left of the illustration a part of the intestinal metaplasia is present. Hematoxylin and eosin. $\times 28$

Figure 10. A high-powered view of an area of atypical glandular lesions is shown in figure 9. The nuclei and cytoplasm are irregular in size and shape. Hematoxylin and eosin. $\times 100$

Figure 11. This is a higher power view of a portion of figure 10. Hematoxylin and eosin. $\times 400$

Figure 12. Cancer-like invasive lesion of the glandular stomach of a rat 59 days after injection of MC (M-I). Proliferation of atypical glandular cells of the pyloric mucous membrane (ulceration) occurring in the submucosal connective tissue. Infiltration of the inflammatory cells can also be seen in this lesion. Mucous membrane to the left. Hematoxylin and eosin. $\times 28$

Figure 13. A high-powered view of atypical glandular structure is shown in figure 12. Mucous membrane to the left. Hematoxylin and eosin. $\times 100$

Figure 14. Cancer-like invasive lesion of the glandular stomach of a rat 198 days after injection of MC and splenectomy (O-III). At the upper right of the illustration a part of the ulceration (regenerated epithelium) is present. Hematoxylin and eosin. $\times 100$

corpus. Infiltration of round-cells and polynuclear leucocytes was observed from the submucosal connective tissue through muscle layer to the serosa.

When the above changes were limited in the submucosa and muscle layer, as a rule, neither breaking through the serosa nor invading to the other organs, or when the cells were atypic, irregular in size and disarranged compared to adenocarcinoma, the histological picture was designated as cancer-like invasive lesion or cancer-like invasion.

(c) Sarcoma was noted in 6 animals at 282, 386, 432 (Figs. 18, 19 and 20), 441, 547 and 486 days, respectively, after the MC-injection. The tumors were all large, hard and round in form. In respect to the mucous membrane, pyloric obstruction and secondary dilatation of the stomach were noted in many of the animals. Gastro-hepatic adhesion was noted to be present in high degrees.

Marked hyperplasia and proliferation of the glandular gastric mucosa were observed. The tumors were generally present from the submucosal muscle layer to the serosa. The nuclei of the tumor cells were oval or irregular in shape, being also variable in size. They generally had scanty nucleoplasm, exhibited coarse chromatin mass, and occasionally one or two distinct nucleoli. The cytoplasm was present in abundance; their shapes were generally oval, round or irregular. Among the atypical cells, giant cells with two or three nuclei were mixed occasionally exhibiting mitotic figures. Some of the tumors showed heavy bleeding and necrosis. Some parts of these tumors consisted of highly polymorphous cells, while the other parts consisted of spindle-shaped cells or long-oval-shaped cells of varying size and shape, and they were arranged in various directions in a complicated appearance, closely resembling that of spindle-cell sarcoma.

Interstitial connective tissue was very scanty, only fine fibrous connective tissue being observed. However, silver-impregnated specimens showed a picture of very abundant argentaffin fibers in proliferation. The above mentioned findings of the 6 animals led to the diagnosis of polymorph-cellular sarcoma.

(d) Ulceration was observed in 82 of the animals belonging to this group. The main

Figure 15. Polymorph-cellular sarcoma of the glandular stomach of a rat 214 days after injection of MC and splenectomy (M-III). Invasion of the sarcoma cells has extended through the submucosal connective tissue into the serosa. Mucous membrane to the above. Hematoxylin and eosin. $\times 28$

Figure 16. A high-powered view of an area of the neoplastic lesion with giant cells shown in figure 15. In the group of strongly atypical cells, giant cells with 2 to 3 nuclei are mixed, and is exhibiting mitotic figures. Hematoxylin and eosin. $\times 100$

Figure 17. A high-powered view of an area of neoplastic lesion with spindle-shaped cells shown in figure 15. Hematoxylin and eosin. $\times 100$

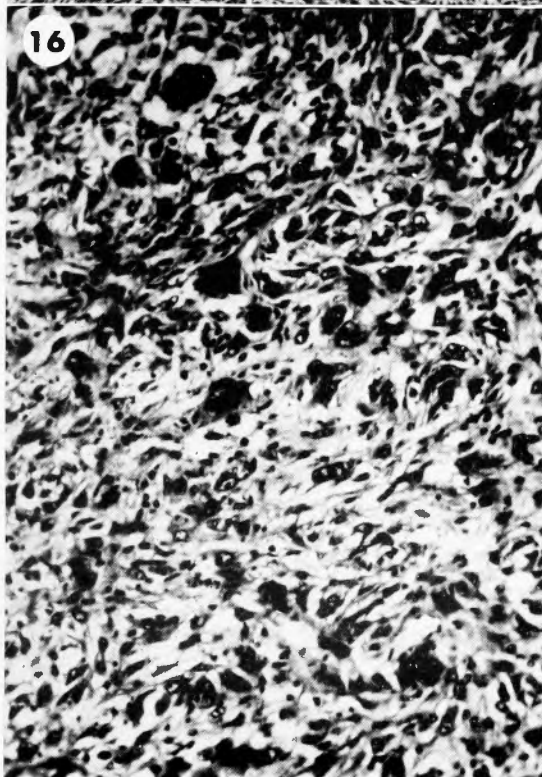
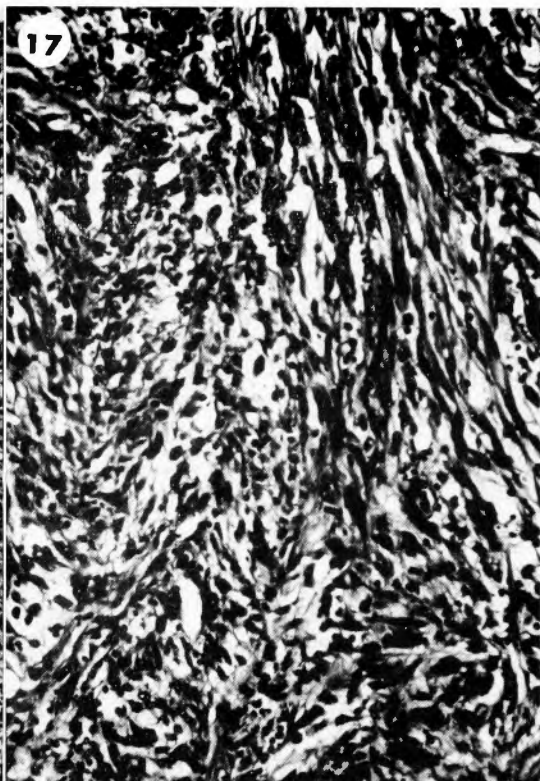
Figure 18. Polymorph-cellular sarcoma of the glandular stomach of a rat 432 days after injection of MC (O-I). This neoplastic lesion occurred in the submucosal connective tissue. The cells are polymorphic. Mucous membrane to the left. Hematoxylin and eosin. $\times 28$

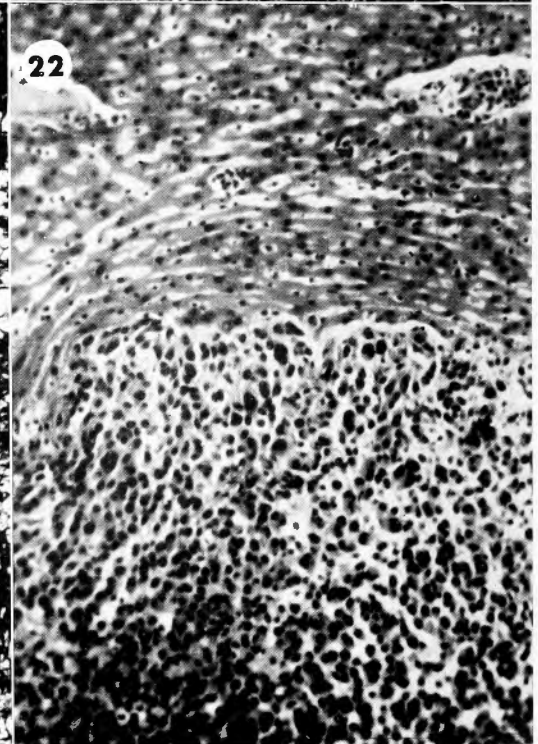
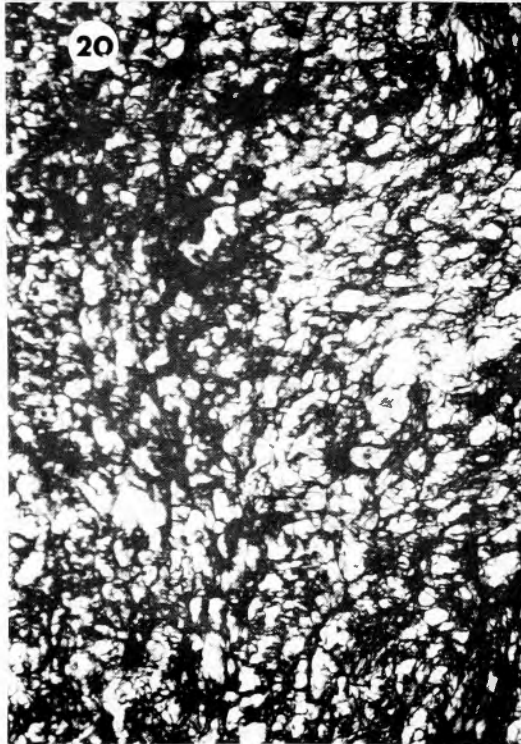
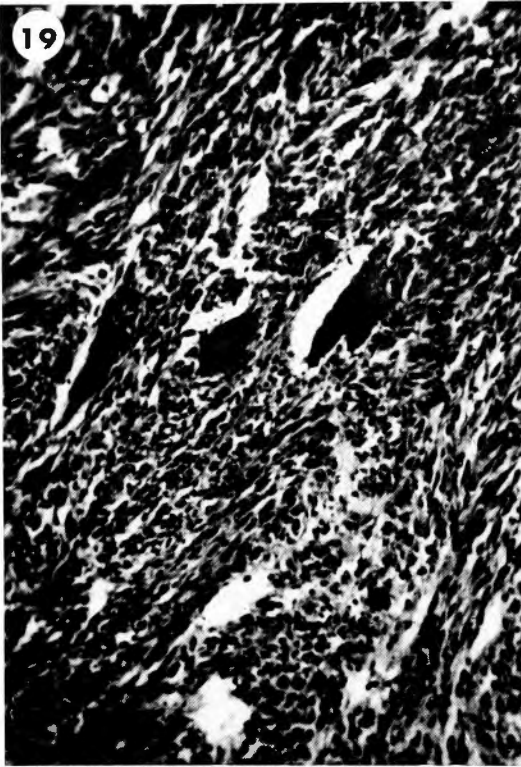
Figure 19. A high-powered view of giant cells shown in figure 18. Hematoxylin and eosin. $\times 100$

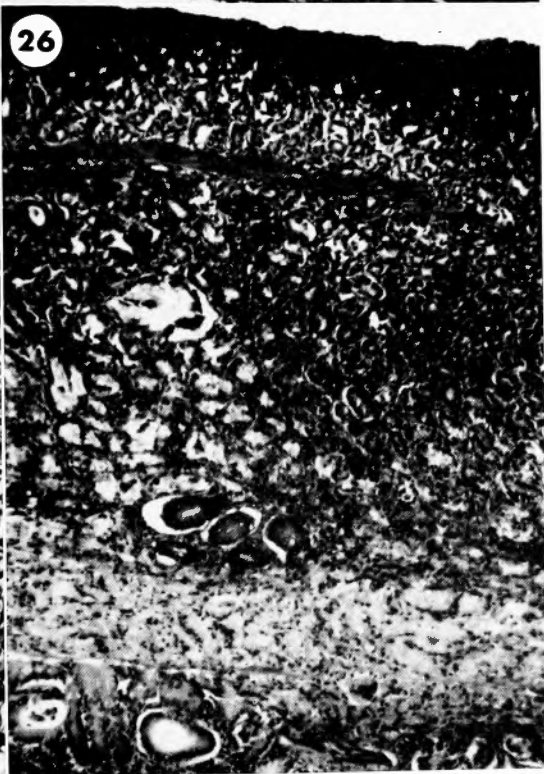
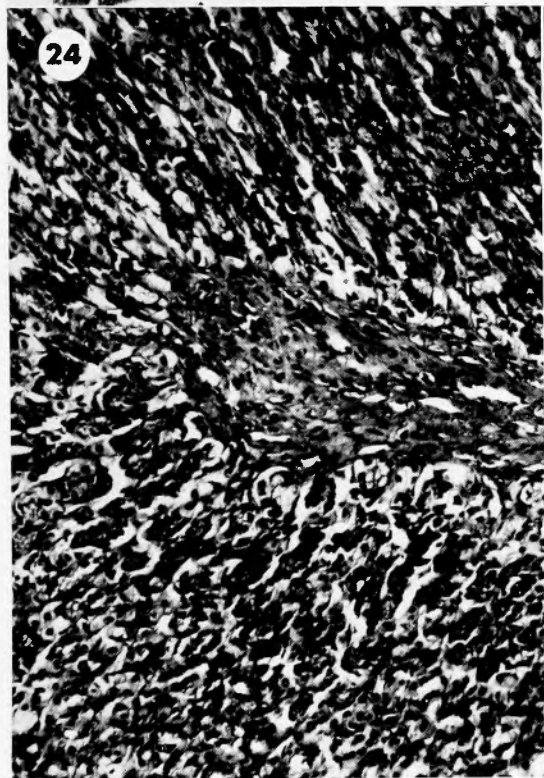
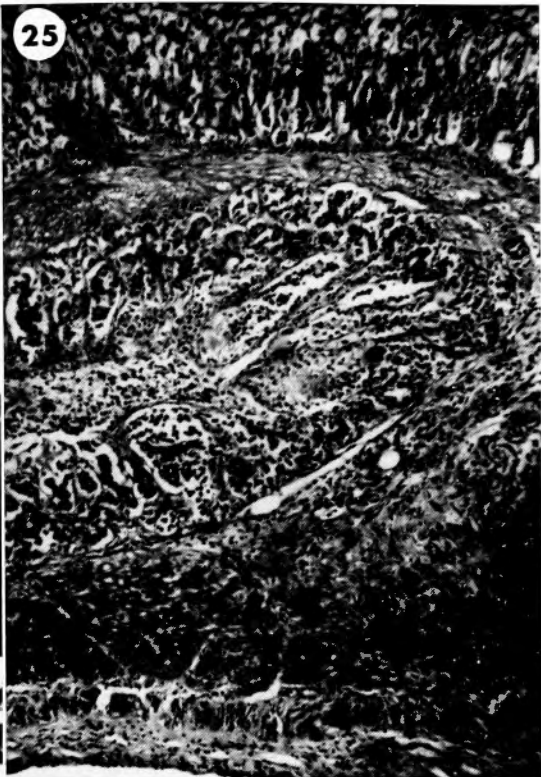
Figure 20. A high-powered view of a portion of figure 18. Remarkable proliferation of an argentaffin fibers is occurring in the interstitial connective tissue. Silver impregnation. $\times 100$

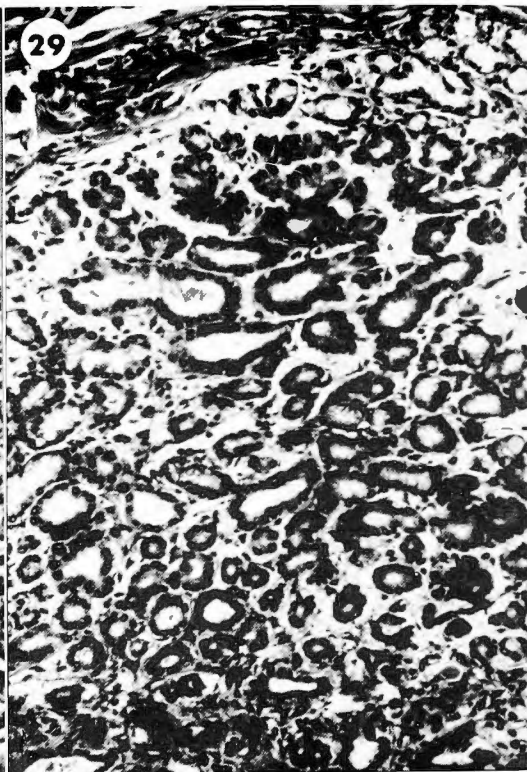
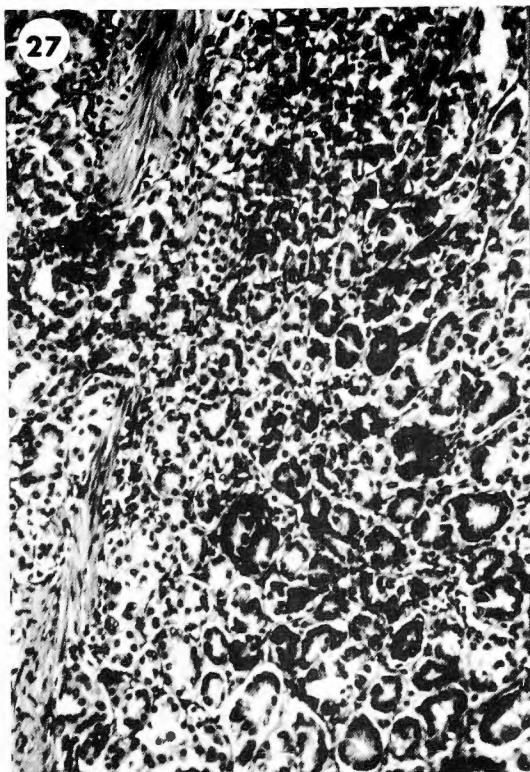
Figure 21. Liver metastasis occurring by polymorph-cellular sarcoma of glandular stomach of a rat 190 days after injection of MC and splenectomy (M-III). The neoplastic lesion is invaded into the intra-hepatic tissue. Liver tissue is in the above. Hematoxylin and eosin. $\times 28$

Figure 22. A high-powered view of a portion of the liver metastasis shown in figure 21. The nuclei and cytoplasm are irregular in size and shape. Hematoxylin and eosin. $\times 100$









change in these animals was defect in the mucous membrane. Some showed pronounced proliferation in glandular structures without being accompanied by atypical proliferation and other malignant changes of the cells. Most of the ulcers developed at the pylorus where MC had been injected, and in some cases, extended to the corpus. The mucous membrane around the ulcers was markedly thickened, showing reactive hyperplasia and partly exhibiting adenomatous proliferation. Some of the ulcers seem to have the nature of tumors. The surface of the ulcers were covered with necrotic tissue. Fibrous, hyalinized connective tissue proliferation was also noticeable. The base of the ulcer was a thick granulation tissue proliferation with small round-cell infiltration. Intense adhesion to the liver was found in many of the cases. Degeneration, atrophy, and loss of liver-cells were observed. The liver-cells exhibiting degenerative atrophy were divided in two or several cell-groups. The bile duct in the hepatic lobule also presented intense proliferation. (e) Adenomatous proliferation or benign adenoma formation was noted in 153 animals (Figs. 23 and 24). The first observed was at 14 days after the MC-injection. The mucous membrane at the site of injection, one or two days after the MC-injection into the submucosa of the glandular stomach, showed mild hyperplasia compared to the normal gastric mucosa.

The fibroblastic proliferation and the round-cell infiltration were observed in high degree from the muscularis propria to the submucosa at the injection area, occasionally making it difficult to discern the glandular structure. Seven to fourteen days after the MC-injection, the glandular cavity between the muscularis propria and submucosa became larger with some decrease in the round-cell infiltration, and the glandular structure was a little more distinct. A part of the glandular structure was found to extend near to the lamina muscularis mucosae. The cytoplasm of the individual cells constituting the glandular cavity was lightly stainable with eosin, cylindrical in form, and had relatively

Figure 23. Adenomatous proliferation of the glandular stomach of a rat 63 days after injection of MC (O-I), showing papillary growth of the glandular epithelium at the pyloric mucosa. Hematoxylin and eosin. $\times 28$

Figure 24. A high-powered view of a portion of figure 23, showing atypicality of the cells and glands. Mucous membrane to the left. Hematoxylin and eosin. $\times 100$

Figure 25. Adenomatous proliferation of the glandular stomach of a rat 177 days after injection of MC (M-II). This shows the glandular structure surrounding by the capsule composed of connective tissue in the fundic muscle layer. Mucous membrane is in the above. Hematoxylin and eosin. $\times 28$

Figure 26. Adenomatous proliferation of other parts of figure 25. Proliferation of the pyloric glandular cells is extended through the muscularis mucosae into the serosa. Mucous membrane is in the above. Hematoxylin and eosin. $\times 28$

Figure 27. A high-powered view of a portion of the glandular structure at the muscularis mucosae shown in figure 26. Mucous membrane to the left. Hematoxylin and eosin. $\times 100$

Figure 28. Adenomatous proliferation of the glandular stomach of a rat 484 days after injection of MC (M-II). Proliferation of glands and cells occurring in the pyloric submucosal tissue. Mucous membrane is in the above. Hematoxylin and eosin. $\times 28$

Figure 29. A high-powered view of the glandular structure at the submucosa shown in figure 28, showing slight atypicality of the glands and cells. Hematoxylin and eosin. $\times 100$

Figure 30. Intestinal metaplasia in the pylorus of the glandular stomach of a rat 399 days after injection of MC (O-I). There is an intestinalization of the epithelium at the pyloric mucous membrane. Hematoxylin and eosin. $\times 100$

indistinct cell contours. Atypcy was not pronounced, and cell arrangement was not so irregular. The nucleus was generally located at the center of the cell, with one or two nucleoli deeply stainable with hematoxylin. There was scarcely any irregularity in the size of the nucleus. Occasionally, the nucleus was somewhat large for the protoplasma, and its shape was also irregular.

Twenty to fifty days after the MC-injection, the glandular structure at the gland-base of the gastric mucosa adjacent to the site of injection, continued to proliferate further and extend to the lamina muscularis mucosae, some proceeding further into the submucosal tissue and proliferate there (Figs. 25 and 26). But, the irregularity in the size of the nucleus and the arrangement of cytoplasm were observed not so often.

Seventy days after the MC-injection, the glandular tissue proliferated through the lamina muscularis mucosae into the submucosal tissue, further to reach the muscularis propria or subserosal tissue. At the same time the connective tissue around the glandular structure was also seen to proliferate.

About 100 days, the adenomatous proliferation was so intense that the glandular structure presented now irregular arrangement and shape in some instances. In these changes the adenoma and the adenomatous diverticulum were included. These epithelial cells were generally cell-groups surrounded by fibrous connective tissue in the submucosal muscle layer. The protoplasma of the individual cells constituting those cell-groups was eosinophilic and had oval or somewhat irregular shape. The nucleus was round and relatively rich in chromatin and possessing one or two distinct nucleoli. These cells constituted glandular structure and formed several cell-groups. There were very few mitotic figures to be seen.

(f) Intestinal metaplasia was observed (Fig. 30). When MC-suspension or -emulsion acted directly upon the stomach wall of rats, the gastric mucous membrane indicated inflammatory changes such as small round-cell and leucocytic infiltration, followed by the ulcer formation and adenomatous proliferation in the glandular portion of the stomach. Then, the epithelial cells regenerated to cover the ulcerated surface. The regenerative epithelial cells at the ulcerative area occasionally exhibited atypical proliferation, which is considered sometimes to persist in that state over a long period of time, and at other times to change eventually into carcinoma cells. Thus, a series of changes, i. e., from normal through ulceration and regeneration (metaplasia) to cancerization, was conceivable. When the carcinogenic agents acted insufficiently in quantity or also in the period of time, cancerization of the metaplastic cells would not have occurred. The carcinogenic agent has an activity to initiate metaplasia of the cells. In the present experiment, intestinal metaplasia was not highly malignant in any of these animals.

(g) Squamous-cell metaplasia was noted in 34 animals, mainly involving the mucous membrane from the bordering part between the forestomach and glandular stomach to the corpus. These squamous epithelia generally exhibited much cornification, presenting much proliferation of prickly cells. This condition was occasionally accompanied by proliferation of submucosal connective tissue and infiltration of polynuclear leucocytes and plasma cells.

2) Group of intramural injection of MC-at the pylorus and corpus, 47 animals (M-II

and O-II) :

This group was given 0.01 cc (0.4 mg) each of 4% MC-suspension or -emulsion at two sites, i. e., at the pyloric portion and fundic portion of the glandular stomach. The results were as follows,

Adenocarcinoma	0
Cancer-like invasive lesion	2 (4.2%)
Sarcoma	2 (4.2%)
Ulceration	19
Adenomatous proliferation and adenoma	30 (24 in pylorus) 6 in corpus)
Intestinal metaplasia	0
Squamous-cell metaplasia	6

(a) As for the malignant changes, there was no evidence of adenocarcinoma, nothing more than the occurrence of cancer-like invasive lesion in two animals and polymorph-cellular sarcoma in two animals.

(b) Adenomatous proliferation was observed at the pylorus in 24 animals and at the corpus in 6 animals (Figs. 25, 26, 27, 28 and 29). The changes were especially conspicuous at the pylorus. This indicates markedly different sensibility to MC between the pylorus and the corpus. The pylorus of the stomach, thus, seemed to be far more sensitive to MC than the corpus, which coinciding with the fact that the gastric lesions in man were found more often at the lesser curvature of the pylorus.

3) Group of intramural injection of MC and ethanol at the pylorus, and splenectomy, 85 animals (M-III and O-III) :

The following changes were observed in this group,

Adenocarcinoma	2 (2.3%)
Cancer-like invasive lesion	25 (28.8%)
Sarcoma	12 (14.1%)
Ulceration	48
Adenomatous proliferaion and adenoma	65
Intestinal metaplasia	5
Squamous-cell metaplasia	8

(a) Adenocarcinoma was observed in two animals, one at 168 days after the MC-injection (Figs. 4 and 5) and the other, 149 days (Figs. 6, 7 and 8). In both animals the preaseace of giant ulcers were noted on the mucosa surface of the corpus and pylorus. Adhesion of high degrees was observed at the anterior wall of the glandular stomach and the liver surface, with conspicuous thickening of the mucous membrane of the glandular stomach. Tumors observed microscopically in the submucosal connective tissue exhibited atypy and irregularity in size and arrangement of the cells.

Cancer-like invasive lesion, adenomatous proliferation and invasion into the liver tissue were noted in some cases. A continuous infiltration of atypical glandular structure beginning on the surface of the ulcer, penetrating the muscularis mucosae and serosa, and proceeding into the liver parenchyma, were observed. This was interpreted to be glandular epithelial cell of the stomach, which might be infiltration of adenocarcinoma into the liver tissue (Figs. 6, 7 and 8).

(b) Twelve animals of this group exhibited sarcoma (Figs. 15, 16 and 17), which like those occurring in the non-splenectomized group was polymorphous sarcoma. Hepatic metastasis was observed in eight of twelve animals (Figs. 21 and 22).

(c) Cancer-like invasive lesion was noted in 25 animals (Figs. 9, 10, 11 and 14). There was not much difference in the histological picture from the changes observed in the non-splenectomized group. The incidence of adenocarcinoma, sarcoma and cancer-like invasive lesion, however, was far higher in the present group than in the non-splenectomized group. The incidence of adenomatous proliferation and intestinal metaplasia which are considered to be precancerous changes also seemed to be a little higher in the present group than in the non-splenectomized group. This may be attributed to the promotion of tumor cell proliferation due to the deficiency in splenic functions caused by splenectomy. Post-operative growth and increase in body weight seems to be much less in the splenectomized group than in the non-splenectomized group. Besides, many of the splenectomized animals died shortly after the operation. This may be due to decrease of general resistance as a result of splenic disfunction (Tab. 4 and 5). As presented in Tab. 3, the metastasis of malignant tumors to the liver seems to appear more frequently in the splenectomized

Table 4

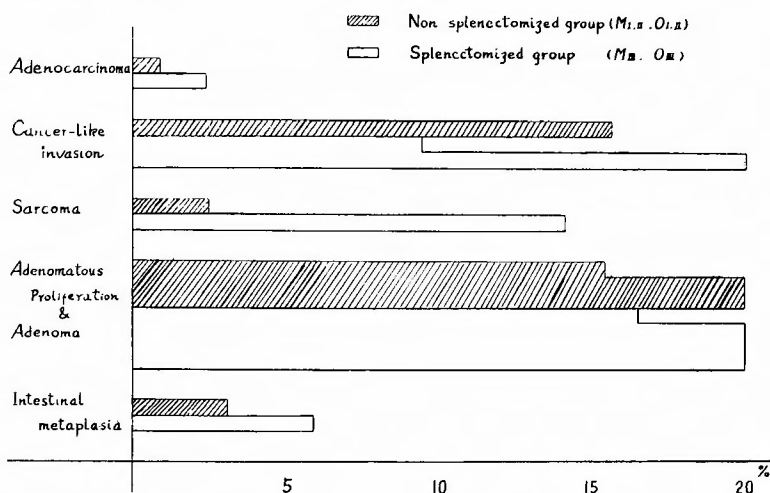
Days post injection	MC-Methocel suspension				MC-Olive oil emulsion			
	M-I	M-II	M-III	Totals (Tumors)	O-I	O-II	O-III	Totals (Tumors)
1~ 29	12	1		13	50	12	9	71
30~ 59	6 ₍₁₎	2		8 ₍₁₎	10		1	11
60~ 89	20 ₍₈₎ ∴ ∴ ∴	8	2 ₍₂₎	30 ₍₁₀₎	10 ₍₁₎			10 ₍₁₎
90~119	17 ₍₂₎	4		21 ₍₂₎	11		2 ₍₂₎ ∴ ∴	13 ₍₂₎
120~149	23 ₍₃₎ ∴ ∴	8	3 ₍₁₎ ∴ ∴	34 ₍₄₎	12 ₍₃₎ ∴ ∴		12 ₍₄₎ ∴ ∴ ∴	24 ₍₇₎
150~179	21 ₍₇₎ ∴ ∴ ∴	5 ₍₁₎ ▲	9 ₍₄₎ ▲ ∴ ∴ ∴	35 ₍₁₂₎	3 ₍₁₎ ○		8 ₍₆₎ ○ ∴ ∴ ∴	11 ₍₇₎
180~209	11 ₍₁₎	2	17 ₍₁₀₎ ▲ ▲ ▲ ▲ ▲	30 ₍₁₁₎	1		12 ₍₇₎ ▲ ▲ ∴ ∴	13 ₍₇₎
210~239	5 ₍₂₎ ○	1	5 ₍₂₎ ▲ ▲	11 ₍₄₎	1		2 ₍₁₎ ▲	3 ₍₁₎
240~269	3 ₍₃₎ ∴ ∴	1 ₍₁₎ ▲	1	5 ₍₄₎	2 ₍₁₎		2	4 ₍₁₎
270~299	2 ₍₁₎			2 ₍₁₎	2 ₍₂₎ ▲			2 ₍₂₎
300~329	3 ₍₃₎ ○			3 ₍₃₎	5			5
330~359	2			2	3 ₍₂₎ ▲			3 ₍₂₎
360~389	2 ₍₁₎	1 ₍₁₎		3 ₍₂₎	1 ₍₁₎ ▲			1 ₍₁₎
390~419	1 ₍₁₎			1 ₍₁₎	1			1
420~449	3 ₍₁₎			3 ₍₁₎	6 ₍₂₎ ▲			6 ₍₂₎
450~479	5 ₍₁₎			5 ₍₁₎	6 ₍₁₎			6 ₍₁₎
480~509	3 ₍₂₎	2 ₍₁₎		5 ₍₃₎	1 ₍₁₎ ▲			1 ₍₁₎
510~539	1			1	1 ₍₁₎			1 ₍₁₎
540~569					2 ₍₁₎ ▲			2 ₍₁₎
570~599	2 ₍₂₎ ∴			2 ₍₂₎	1 ₍₁₎			1 ₍₁₎
600~629	1			1				
Totals	143 ₍₃₉₎ ○ 2 • 37	35 ₍₄₎ ▲ 2 • 2	37 ₍₁₉₎ ▲ 8 • 11	215 ₍₆₂₎ ○ 2 ▲ 10 • 50	129 ₍₁₈₎ ○ 1 ▲ 6 • 11	12 ₍₀₎	48 ₍₂₀₎ ○ 2 ▲ 4 • 14	189 ₍₃₈₎ ○ 3 ▲ 10 • 25

○ Adenocarcinoma • Cancer-like invasion ▲ Sarcoma () Number of malignant tumors

group.

In the present experiments, there was no great difference in the incidence of malignant tumors between the methocel-suspension group and the olive-oil-emulsion group.

Table 5



DISCUSSION

Attempts to produce adenocarcinoma experimentally in the stomach of mice and rats, which seldom shows spontaneous growth of stomach cancer (8, 54, 62), were first reported by WATERMAN (1936)⁶¹, ROFF (1938)⁴⁵, etc. They were able to produce squamous-cell carcinoma in the forestomach by oral administration of cholesterol or heated oil to rats, but, they could not produce adenocarcinoma. Many other experiments were made subsequently by oral administration of various carcinogenic substances (mixed oil, tar mixture, aniline oil and toluylene-diamine etc)⁶⁰. Most of these experiments produced lesions in the forestomach, but very rarely produced adenocarcinoma.

Carcinogenic agents are thus known hardly able to give rise to gastric adenocarcinoma in rats by oral administration. This phenomenon was explained by HOLLANDER (1944)^{18,19}, IVY (1945)²⁰, STEWART (1953)⁵², etc. as follows: The glandular stomach is covered with the mucous membrane and its epithelia are more resistant than those of the forestomach. Furthermore, orally administered carcinogenic agent may flow out of the glandular stomach rapidly without doing much harm on it. Therefore, several attempts were made, to keep the carcinogenic agent in direct contact with and to act upon the mucous membrane of the glandular stomach for a long periods of time in order to induce gastric carcinoma with it.

HARE, STEWART, BENNETT and LORENZ¹⁷ injected 0.02 cc of 3% aqueous suspension of MC (containing 0.6 mg of MC) into the submucosal tissue of the glandular stomach of rats and produced adenocarcinoma in 4 of 265 animals (1.5%) after 11 to about 35 months. The author employed a technique similar to the method by STEWART et al.^{17, 28, 47, 48, 49, 50, 53}. That is, 0.01 cc of 4% aqueous suspension or emulsion of MC and 0.01 cc

of 90% ethanol were injected into the submucosa of the glandular stomach of rats. Production of adenocarcinoma were in 2 of 85 splenectomized animals and also in 3 of 319 non-splenectomized animals by this method.

Adenomatous proliferation induced by intramural injection of MC was presumed by STEWART, HARE, BENNETT and LORENZ (1952)¹⁷⁾, MORI et al. (1955)^{33, 34)} to be a precancerous lesion, and also in the present author's preparations, similar cytological changes were observed. KONJETZNY^{23, 24)} and MOSZKOWICZ³⁶⁾ regarded importance to the regenerative process of the epithelium in gastritis or gastric ulcerative lesions. OOTA⁴³⁾ stated that normal gastric epithelium could convert systematic metaplasia into an accessory cell, or into an epithelium of gastric foveola, or into an intestinal epithelium, and that any of these cells can metaplasiate into an indifferent cell. Further, he considered that there is a close relation between metaplasia and cancerization. MURAKAMI^{30, 31, 32)} and MORSON^{37, 38)} pointed out a transforming point from metaplasia of an indifferent cell or intestinal epithelium into gastric carcinoma, and they suggested this point to be the origin of cancer. It has recently been increasingly ascertained with progress of studies on early tumors that metaplasia takes place prior to cancerization.

MORI et al. (1955)³⁵⁾ administered MC to rats by the oral route and produced adenomatous proliferation with intestinal metaplasia, which they recognized to be a step of cancerization. In the present experiment, intestinal metaplasia was observed in ten of the non-splenectomized animals and in five of the splenectomized animals.

HARE, STEWART, BENNETT and LORENZ (1952)¹⁷⁾ injected the same dosage of MC simultaneously into the fundic and pyloric portion of the glandular stomach wall in rats and noted higher incidence of adenocarcinoma and adenomatous proliferation at the pyloric portion. According to them, this is similar to the fact that gastric lesions in man occur more frequently in the pylorus than in the cardiac portion of the stomach. They emphasized difference in the sensibility to carcinogen between the fundic and pyloric portion of the stomach. The present author's experiments also found such lesions as adenocarcinoma, sarcoma, cancer-like invasive lesion, ulceration and adenomatous proliferation occurring much more frequently at the pyloric region.

It is often difficult to diagnose histologically whether experimentally produced hyperplasia of gastric glandular epithelia is benign or malignant. Apart from instances in which metastasis to lymph nodes and to other organs is evident, it is dangerous to diagnose a heterotropic, atypical proliferation to be a cancer. STEWART et al.⁵¹⁾ were very cautious in the judgement of these lesions, and they instituted the following criteria for histological diagnosis. They indicated that the term "carcinoma" implies the following conditions: namely, the epithelial neoplastic cells have infiltrated all coats of the stomach wall and extended onto the serosa. The term "precancerous lesion" was defined to indicate the following conditions i. e., atypical neoplastic cells are observed in the gastric mucosa, submucosa and muscularis mucosae, but not reaching the serosa and not being accompanied by metastasis. The present author considers precancerous lesions in its broad sense as adenomatous proliferation and intestinal metaplasia.

It has long been known since the reports of BIELING⁴⁾, ASCHOFF¹⁾, NEUFELD¹¹⁾, SINGER and ADLER⁵⁵⁾ (1923-1924), that the spleen participates in the production of antibodies

and thus plays an extremely important role in onco-immunity. It has been demonstrated experimentally and clinically by LUBARSH²⁸⁾, BRAUNSTEIN³⁾, DI BIASI¹⁾, YOKOHATA⁶⁵⁾ (1926-1930) and others that malignant neoplasms, primary or secondary, occurs very rarely in the spleen. BRÜDA⁷⁾, BRAUNSTEIN⁵⁾, APOLANT²⁾, OSER and PRIBRAM⁴⁴⁾, TANAKA^{58,59)} and SHIMURA et al.⁵⁴⁾ reported on the effect of the spleen upon the proliferation of neoplasms and concluded that splenic insufficiency promotes proliferation of tumors. Splenic insufficiency was reported by AZUMA et al.³⁾ to inhibit tumor proliferation.

BISCHOFF and MAXWELL⁹⁾, ROHDENBURG⁴³⁾, MEYER⁴⁰⁾, FUJINAWA¹⁵⁾ said that there was no effect upon the tumor proliferation. A recently released report stated that the reticulo-endothelial system has activity against spontaneous neoplasms and malignant tumors induced by chemical substances. IWASE and FUJITA^{21,39,57,33)} stated that, in liver cancer produced in rats by means of azo-dye, pretreatment with trypan-blue injection lessens the amount of protein-bound dye in the liver tissue than in the controls, delays the production of the tumor, and makes the degree of malignancy lower. The difference observed above were believed by IWASE and FUJITA to be ascribable to the early enlargement of the cells of the hepatic reticulo-endothelial system, and also to the proliferation of the reticulo-endothelial cells and histiocytic cells due to the administered trypan-blue.

It was also found by the clinical experience that, in the total gastrectomy performed on patients with gastric cancer, the extirpation of the regional lymph nodes often makes it unavoidable to carry out splenectomy.

As compared with total gastrectomy alone, the gastrectomy with concomitant splenectomy is known to bring about earlier deterioration of the patient's general conditions and earlier recurrence of neoplasms. It is reported that there can be seen the appearance of more undifferentiated cell types in recurrence than in the primary lesion. The rapid exacerbation in general conditions and the extremely rapid progress in neoplastic proliferation may be considered to be an outcome of splenectomy. All these facts suggest the presence of defensive mechanism in the spleen and reticulo-endothelial system against malignant tumors.

The most important thing in the management of gastric cancer at present is a radical operation involving extirpation of the regional lymph nodes. This surgical management is often performed at the sacrifice of the spleen. In order to inhibit neoplastic growth and to reinforce surgical management, concomitant use of carcinostatic agents or radiation therapy is carried out as a recent tendency. For better results of gastric cancer treatment and for prophylaxis of its recurrence, the relationship between gastric neoplasms and spleen or reticulo-endothelial system has to be understood more profoundly than it is at present¹³⁾.

In the present experiment, the incidence of gastric malignant tumors including the lesions above mentioned, was much higher in the splenectomized group than in the non-splenectomized group. Inhibition of post-operative growth and shorter survival were observed more distinct in the splenectomized group (Tab. 4 and 5).

CONCLUSION

In order to establish what factors are responsible for the production of malignant gastric neoplasms experimentally, author performed experiments with Wistar-strain rats

which are known to have a low incidence of spontaneous gastric cancer. The present experiment was performed by the following techniques i. e., injection of carcinogenic agent MC into the submucosal tissue of the glandular stomach, blocking of the reticulo-endothelial system by splenectomy, and use of various solvents of MC, local injection of ethanol, etc.

The results obtained were as follows :

(1) Malignant changes were observed in 61 of 319 animals not subjected to splenectomy. The 61 animals consisted of 3 with adenocarcinoma, 8 with sarcoma and 50 with cancer-like invasive lesion. Precancerous changes were noted in 193 animals, consisting of 183 with adenomatous proliferation and 10 with intestinal metaplasia. In contrast to the above, malignant changes were observed in 39 of 85 animals subjected to splenectomy, consisting of 2 animals with adenocarcinoma, 12 with sarcoma and 25 with cancer-like invasive lesion. Precancerous changes were noted in 70 animals, consisting of 65 animals with adenomatous proliferation and 5 with intestinal metaplasia.

The figures quoted above indicate that malignant changes and precancerous changes had far higher incidence in the splenectomized group than in the non-splenectomized group. Liver metastasis was observed in 8 animals with sarcoma and in 2 with adenocarcinoma of the splenectomized group, and in only 2 animals with adenocarcinoma of the non-splenectomized group. Besides, the post-operative body growth was generally poorer and survival shorter in the splenectomized group.

These findings indicated that splenectomy potentiate carcinogenesis, proliferation of tumors and metastasis. This unfavorable result seems to be ascribable to decrease in general resistance of the animals subjected to splenectomy.

(2) As for the variety of solvents, the incidence of malignant gastric neoplasms was not found particularly different between the group of methocel-suspension and the group of olive-oil-emulsion.

(3) The author's experiments have shown that malignant and precancerous changes occur more often in the pyloric than in the fundic portion of the glandular stomach.

(4) In some of the animals exhibiting intestinal metaplasia, pictures of atypical growth of cells were observed. This may be taken to endorse the general interpretation of intestinal metaplasia as a precancerous lesion.

The author wishes to express sincere gratitude to Dr. RYO INOUE, an instructor of the surgical clinic, for many valuable suggestions and helpful discussions in this investigation. The author is also very grateful to Dr. YASUYUKI NISHIZUKA, professor of the pathological division of Mie Medical College, for his kind guidances throughout this work.

The author's grateful thanks are due to Drs. YOSHIYUKI YOSHIDA and SHIRO NAKAMURA, members of the surgical clinic, and also Dr. AKIRA NAKAGAWA, an instructor of the pathological division of Kobe Medical College, for their helpfulness in the course of the present work.

The gist of the present study was reported at the 21th General Meeting of the Japanese Cancer Association at Tokyo (October 1962).

The work reported in this paper was supported in part by Cancer Research Grant from Nippon Life Insurance Company (Osaka, Japan), and also by Medical Research Grant from Japanese Ministry of Welfare.

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(*Written in Japanese)

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実験的胃悪性腫瘍の発生に及ぼす細網内皮系の
影響に関する研究

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現在胃癌に対して所属淋巴節を含む広範な手術療法が最も重要であり、その際脾摘が行われる事も多いのであるが、他方、生体の防禦機能を司る脾、淋巴腺等の網内系の遮断に依る生体の抵抗力減弱の点も考慮しなければならない。そこで癌原物質 Methylcholanthrene (MC) を含む水性浮游液或は乳剤を Wistar 系白鼠の腺胃粘膜下組織に注入し更に Ethanol の局所同時注入、或は網内系遮断 (脾摘) 等の処置を併用し、実験的胃悪性腫瘍の発生に如何なる因子が関与するものかを追求する目的で次の実験を行った。即ち、4% MC-Methocel-Suspension 群 (M群) と 4% MC-Olive-Oil-Emulsion 群 (O群) とに大別し、更に次の様な処置に依り 6 群に分った。実験有効動物数は 404 匹であった。

(I) 4% MC 水性浮游液 0.01cc と 90% Ethanol 0.01 cc を同時に 腺胃幽門部粘膜下組織内に 注入した群 (M-I : 143匹)。

(II) 4% MC オリーブ油乳剤 0.01 cc と 90% Ethanol 0.01cc を同時に幽門部粘膜下組織内に注入した群 (O-I : 129匹)。

(III) 4% MC 水性浮游液 0.01cc 宛を幽門部及び体部に各々注入した群 (M-II : 35匹)。

(IV) 4% MC オリーブ油乳剤 0.01cc 宛を幽門部及び体部に各々注入した群 (O-II : 12匹)。

(V) 4% MC 水性浮游液 0.01 cc と 90% Ethanol 0.01cc を幽門部に注入し、更に脾摘を併せて行った群 (M-III : 37匹)。

(VI) 4% MC オリーブ油乳剤 0.01cc と 90% Ethanol 0.01cc を幽門部に注入し脾摘を併せて行った群 (O-III : 48匹)。

上記の各処置群を経時的に肉眼的、組織学的に検索し次の様な結果を得た。

(1) 非脾摘群319匹中、61例に悪性変化 (腺癌 3. 肉腫 8. 癌様浸潤 50) を、193例に前癌性変化 (腺腫様増殖 183. 腸上皮化生 10) を来たした。これに対し、脾摘群85匹中39例に悪性変化 (腺癌 2. 肉腫 12. 癌様浸潤 25) を、70例に前癌性変化 (腺腫様増殖 65. 腸上皮化生 5) を得た。これに依って脾摘群に於ては悪性変化及び前癌性変化の発現が非脾摘群に比較して遙かに高い事が判明した。又腺癌、肉腫の転移に就いても同様で、非脾摘群では全く肝転移がみられなかった。更に、脾摘群では一般に術後の成長が阻害せられ、生存期間が短かった。此の事は結局、脾摘に依る脾臓機能の脱落の為に全身抵抗力の低下を来たし、腫瘍の発生及び増殖転移を促進せしめる結果に基づくものと推察される。

(2) 溶剤の種類に就いては Methocel-Suspension 群と Olive-Oil-Emulsion 群との間に悪性腫瘍の発生率に大差は認められなかった。

(3) 悪性変化及び前癌性変化が胃体部に比較して幽門部に多く認められた。

(4) 腸上皮化生を来たしたものの中には異型細胞増殖を示すものが認められた。