Infiltrating Adenomatous Lesions of the Stomach, Cecum, and Rectum of Monkeys Similar to Early Human Carcinoma and Carcinoma in situ∗

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The experimental production in animals of neoplastic gastro-intestinal lesions of types common in man has proven extremely difficult and, in the case of most lesions, rarely successful. Spontaneous gastro-intestinal diseases in animals, clinically and pathologically similar to those of man, are also quite unusual. Only recently has experimental carcinogenesis in the intestine of rodents been successful with sufficient constancy to be of aid in the study of the pathogenesis of cancer in this site (1, 2). Even so, few adenocarcinomas of the stomach (3, 4), colon (2), and rectum of animals have been produced experimentally. Therefore the repeated, facile production of carcinoma-like lesions in the stomach (5), colon, and rectum of monkeys who had ingested motor lubricating oil is of great interest. The novelty of these findings in this species is affirmed by the few spontaneous carcinomas that have been reported in monkeys (6, 7) and by the apparent resistance of monkeys to the action of carcinogens even through 10 years of continuous treatment (7).

The observations recorded here extend those previously made, reporting the occurrence in Macaca mulatta (rhesus) monkeys of a hyperplastic gastritis characterized by invasion of the submucosa by proliferating hyperchromatic glands (5). These lesions were similar to those produced by Bonne and Sandground (8) in Javanese monkeys by a parasitic nematode. No gastric parasites, however, were found in the lesions in the rhesus monkeys and the responsible etiological agent in these cases seemed to be an oil to which the monkeys were exposed for several months. The impression that this oil was derived from shale and contained substances similar chromatographically to known carcinogens was corrected recently by a careful chromatographic study of this oil using more refined instruments and techniques (9). The oil, a common diesel motor lubricant, used by the Chemical Corps, U.S. Army, for producing smoke screens, was found to be free of any known carcinogenic substances. The gastric lesions previously described (5) were found unexpectedly in the course of experiments investigating the dangers of inhaling mists of the oil. The etiological role of the oil could only be assumed in the absence of any knowledge of the spontaneous occurrence of this lesion. Before additional monkeys became available for study of the effect of the oil specifically on their gastro-intestinal tract, rats were fed this oil in synthetic diets. This procedure resulted in the production of a hitherto undescribed adenomatous lesion of the colon of the rats that was quite similar to that in the stomach of the monkeys in appearance and in the manner in which the hyperplastic glands invaded the submucosal tissues (9).

The observations reported here deal with (a) the spontaneous occurrence of this type of lesion in rhesus monkeys, (b) two additional methods by
which the lesion can be produced, (c) the role of the oil and the diet in its production, and (d) the nature of the lesions.

EXPERIMENTAL PROCEDURES

Thirty-six rhesus monkeys weighing between 4 and 5 kilograms were used in these experiments. Three experiments were performed: in the first, 22 monkeys used by other investigators for various purposes were necropsied and the gastro-intestinal tracts carefully investigated for the presence of any disease. All abnormal appearing areas of the stomach, colon, and rectum were taken for microscopic study along with sections removed routinely in all cases. In the stomach the following sites were routinely studied: the pyloric antrum and cardia along the lesser curvature; the junction of the cardia and fundus and of the fundus and antrum along the greater curvature; and fundus from the posterior and anterior surfaces. Other routine sections were: cecum, ascending colon, cecum, and ano-rectal mucosal junction. All tissues were fixed in formalin, imbedded in paraffin and stained with hematoxylin and eosin.

The second experiment comprised an attempt to reproduce the lesions originally found in monkeys exposed to aerosols of oil. Since it was felt that this lesion was the result of the oil being ingested from their bodies during the constant grooming that is done by this species, 10 ml. of oil were sprayed onto the chest and abdomen of 5 monkeys daily until the animals became moribund, a period ranging from 100 to 213 days. Five other monkeys served as untreated controls and were killed simultaneously with the moribund treated monkeys. Microscopic sections were taken and prepared as in the previous experiment.

In the third experiment 4 monkeys averaging 3.3 kg. were used. Instead of the usual diet of dog chow and cabbage, these monkeys received synthetic diets. Two monkeys ate the normal diet and the other two ate a diet containing no protein in order to determine whether protein inanition which accompanies intoxication with the oil could alone produce the hyperplastic infiltrative gastro-intestinal lesions. After 6 months on these diets, the former animals had gained one kilogram each and the other two had lost a similar amount. One of the latter was moribund at that time and was killed and necropsied. The other monkey on the protein-free diet was then placed on the normal diet and the monkeys on that diet changed to the protein-free diet to which 0.83 ml. of oil were added per 100 grams of diet. One of the monkeys ingesting the oil diet died in 48 days and the other died in 78 days after losing 0.7 kg. and 1.2 kg. respectively. The remaining monkey on the normal diet gained 1.6 kg. in 78 days and was then killed and necropsied. Dietary consumption could not be followed in this experiment because the monkeys scattered the crumbly diets widely.

RESULTS

Incidence of gastro-intestinal disease in monkeys not ingesting oil.—Of the 22 monkeys examined, all had normal intestines and 18 had entirely normal stomachs. Seven had varying degrees of atrophic gastritis which in 5 cases was focal and minimal. In 2 cases only occasional parietal cells could be found, the mucosa was thin, and the glands were separated by a lamina propria rich in lymphocytes and containing an increased number of follicles. One apparently normal monkey that in another experiment had lived for over one year in a mist of triethylene glycol had a nodular abnormality of the fundal mucosa. Microscopically this portion of the stomach contained in a fibrotic submucosa large cystic glandular spaces with papillary projections (Fig. 1). The overlying mucosa was relatively normal. A stain for lipase indicated that these structures were composed of normal mucous neck and zymogen-containing cells. These glands were in continuity with the surface crypts. Another monkey that was ill for 3 months following the implantation of an iron ring beneath the scalp in another experiment died with a constricting lesion of the gastric antrum. Microscopically there were cystic glands in the basilar portions of an extremely hyperplastic but simplified mucosa. These cystic glands penetrated the muscularis mucosae in numerous places and formed multiple large cysts in the submucosa (Fig. 2). The external muscular layers were not invaded. The borders of the lesion, which grossly measured 4 cm. in length and encircled the stomach, were composed of hyperplastic epithelial cells that thickened the normal rugae. In these areas occasional examples of early penetration of the submucosa were found (Figs. 3 and 4). Only one monkey had demonstrable intestinal parasites and the gastro-intestinal tract of this animal was normal.

The effect of ingesting lubricating oil placed on the skin.—The 5 control monkeys in this experiment had entirely normal gastro-intestinal tracts. The 5 animals squirted daily with oil had various mucosal abnormalities. None was entirely normal. Monkey 1, dead in 100 days, had an atrophic gastritis (Fig. 5) and the anorectal junction had thickened epithelial folds composed of glands in which the cells were crowded and relatively hyperchromatic. No invasion of the submucosal structures was found. Monkey 2, dead after 120 days, had a hyperplastic gastric mucosa composed
FIG. 1.—Photomicrograph of a portion of the gastric lesion in a monkey that lived about 1 year in an atmosphere containing triethylene glycol, showing large submucosal nests of cystic glands with papillomatous processes. Gomori’s hematoxylin, eosin lipase stain. Mag. X18.

FIG. 2.—Photomicrograph of the central portion of the obstructing lesion in the pyloric antrum of a monkey ill without apparent cause for 3 months, showing hyperplastic epithelium with cystic glands penetrating and filling the submucosa. Hematoxylin and eosin stain. Mag. X13.
FIG. 3.—Photomicrograph of the fundal border of the lesion shown in Fig. 2, showing the hyperplastic mucosa with early penetration of the submucosa (arrow, left) and normal epithelium (arrow, right). The rugal folds are thickened by the mucosa alone. Mag. ×13.

FIG. 4.—Photomicrograph of the pyloric border of the lesion in Fig. 2, showing the hyperplastic gastric mucosa extending to the duodenum. Mag. ×10.
chiefly of mucus-secreting glands (Fig. 7), numerous minute gastric ulcers, and many foci where the muscularis mucosae was penetrated and the edematous, fibrotic submucosa was invaded by cystic glands (Fig. 9). This lesion was identical with those previously reported (5), and the similarity to giant hypertrophic gastritis of man was quite pronounced (Fig. 8). Oil was found in macrophages in hyperplastic perigastric lymph nodes in this monkey. This animal also had a focal submucosal edema in which the submucosa was invaded by penetrating hyperplastic glands in two small areas. In monkey 3, dead after 165 days of the oil, the entire gastric mucosa was abnormal. There was little submucosal fibrosis and only occasional instances of penetration of the muscularis mucosae by surface epithelium. No parietal or zymogenic cells were recognizable. The entire epithelium seemed to be replaced by mucus-secreting cells or poorly differentiated hyperchromatic glands with crowded nuclei. These glands were irregular in size, tortuous, disorderly in arrangement and imbedded in a fibrous lamina propria (Fig. 10). The colon and rectum of this animal were normal. Monkey 4, dead after 175 days, had only partial atrophy of the gastric parietal cells and simplification of the gastric mucosa but the rectum was quite abnormal. Here there were numerous acute superficial ulcerations in the hyperplastic mucosa which was focally heavily infiltrated by polymorphonuclear leukocytes. In many places a few hyperplastic glands invaded the apparently normal submucosa. Monkey 5, dead after 213 days, with stomal gangrene, had a stomach in which the fundus and cardia showed atrophic gastritis. In the cardia, the simplified glands were tortuous and penetrated the muscularis mucosae in numerous areas. The rectal mucosa was extremely hyperplastic and invaded the submucosa extensively. As in the rat lesions with this oil (9) the penetrating glands often contained bacteria and leukocytes or formed abscesses in which the epithelium was necrotic. Large necrotic fissures and diverticula penetrating all the muscular coats of the rectum appeared to be the end result of this process (Figs. 14 and 15).

The effect of protein starvation and ingestion of oil on the gastro-intestinal tract.—Six months of a non-protein diet without the oil did not alter the mucosa of the gastro-intestinal tract of one monkey, but submucosal edema was prominent in the colon of this animal. The monkey that died after 48 days on the non-protein diet containing lubricating oil had an abnormal stomach, cecum, and adjacent colon. There were focal atrophic and hyperplastic mucosal changes in the stomach with a few instances of penetrating glands. In the cecum and colon there were several hemorrhagic, flat, stiff ulcers surrounded by edematous, hyperplastic mucosa. Microscopically these areas were covered by a leukocytic exudate and a low glandular epithelium in which the cells were quite hyperchromatic. Many bottle-shaped deeply penetrating glands were present in the fibrotic submucosa (Fig. 16). In other areas the invading glands were small, irregular in size and shape, and deeply stained with hematoxylin (Fig. 17). The gastro-intestinal tract of the monkey that ate the non-protein diet without oil for 6 months was then changed to the normal diet for 78 days was normal. The monkey that died after 78 days on the non-protein diet and oil diet had severe hyperplastic gastritis in which almost the entire mucosa was composed of mucus-secreting glands (Fig. 12). These glands contained tall columnar cells except in their deepest portions where the acini were crowded with hyperchromatic, poorly differentiated cells (Fig. 13). In foci the process of replacement of the normal epithelium by the mucus-secreting cells appeared to be still in progress. In such areas the proliferating acini formed boat-shaped glands as they grew against the resistance of the submucosa (Fig. 11).

DISCUSSION

These experiments demonstrate that infiltrating adenomatous lesions of the rhesus monkey can be produced with a high percentage of success by feeding the animals this type of motor lubricating oil. They also show that following the treatment the same type of change occurs in the colon and rectum of this species. The two instances where the gastric submucosa was invaded by cystic mucosal glands in monkeys who were not in contact with the oil indicate that this oil is not the only agent which will produce these changes. Evidently, the gastric and colonic mucosae of the rhesus monkey are extremely reactive to non-specific irritative substances. Starvation alone, and particularly protein-starvation, did not produce these reactive changes. The extremely short induction period for these changes when the animals are fed a non-protein diet along with the oil may be indicative of an accelerating effect of generalized lowered resistance to injury. The microscopic similarity of the many instances of atrophic and hyperplastic gastritis to these conditions in man appear to indicate that the mucosa of the monkey reacts similarly to that of the human stomach and colon. These experiments contrast with those of Pfeiffer and Allen (7) who found extremely high resistance of this species to irritative and carcinogenic agents.

By the usual histological criteria used to diag-
FIG. 5.—Photomicrograph of atrophic gastritis in the fundus of a monkey dead after being sprayed with diesel lubricating oil for 100 days. Mag. reduced from ×120.

FIG. 6.—Photomicrograph of a cystic intramucosal focus of hyperplastic mucus neck glands in the fundus of a monkey that ingested the oil in a synthetic non-protein diet for 48 days. Mag. reduced from ×125.

FIG. 7.—Photomicrograph of the stomach of a monkey sprayed daily for 140 days with the oil, showing hyperplastic simplified epithelium composed predominantly of mucous-secreting cells. Compare with Fig. 8. Mag. reduced from ×30.

FIG. 8.—Photomicrograph of the fundus of a human stomach removed because of giant hypertrophic gastritis. Hematoxylin and phloxin stain. Mag. reduced from ×32.
FIG. 9.—Photomicrograph of another area in the stomach of the monkey depicted in Fig. 7, showing the hyperplastic mucosa, an ulcer with partial rep epithelialization, penetration of the muscularis mucosae and infiltration of the submucosa by cystic glands, and edema and fibroplasia of the submucosa. Mag. reduced from X30.

FIG. 10.—Photomicrograph of the gastric mucosa of a monkey sprayed with the oil for 105 days, showing the hyperplastic, hyperchromatic disorderly glands, a lesion compatible in man with carcinoma in situ. Mag. reduced from X56.

FIG. 11.—Photomicrograph of the gastric mucosa of a monkey that ingested the oil in a non-protein diet for 78 days, showing active replacement of the normal epithelium by mucus-secreting glands and the horizontal growth of the abnormal glands along the muscularis mucosae. Mag. reduced from X82.
FIG. 12.—Photomicrograph of another area in the stomach in Fig. 11, showing the cystic, hyperplastic, simplified mucosa with chronic inflammation and the change in the crypts to small, poorly differentiated hyperchromatic acini with crowded cells. Mag. reduced from ×20.

FIG. 13.—Photomicrograph of an area in Fig. 12 in greater detail. Mag. reduced from ×82.

FIG. 14.—Photomicrograph of the rectum of a monkey sprayed on the chest with the oil for 213 days, showing a diverticulous abscess penetrating all layers except the subperitoneal connective tissue. Mag. reduced from ×11.

FIG. 15.—Photomicrograph of another area in the rectum in Fig. 14 but nearer the anus, showing another necrotic fissure bordered by hyperchromatic cystic glands that have invaded the submucosa. Mag. reduced from ×30.
Fig. 16.—Photomicrograph of the cecum of a monkey that ingested the oil in the non-protein diet for 48 days showing an intense cecitis and infiltration of the fibroplastic submucosa by cystic and hyperchromatic glands. Mag. X56.

Fig. 17.—Photomicrograph of another area in the colon in Fig. 16 showing other examples of invading hyperplastic glands in the fibroplastic submucosa and an early abscess in one of these. Mag. X30.
nose malignancy many of these lesions would be called cancerous. Heterotopia, hyperchromasia, poor differentiation, and disorderly cell growth are all present. The lesion in monkey 3 (Fig. 10) is morphologically compatible with the diagnosis of gastric carcinoma in situ. The lesions in the colon of the monkey fed oil and a non-protein diet for 48 days are by these criteria early invasive carcinomas (Figs. 16 and 17). Experimentally, however, these lesions cannot be classified as malignant neoplasia. Inflammation and infection appear to play as great a role in the production of these lesions as does the oil. Acute and chronic inflammatory exudates are usually present in these lesions. In the previous report (5), one monkey, that clinically was suffering from the same diseases as the others that died with infiltrating hyperplastic adenoma-like lesions of the stomach, was saved through diligent nursing care. It was killed about one and a half years later. At that time only a severe atrophic gastritis was present in this animal. In the experiments with rats (9) in which many of the lesions appeared just as malignant as these, the production of the lesions in the presence of the oil was prevented by the concomitant feeding of succinylsulfathiazole. Occasional examples of the rat lesions also showed areas in which the process had subsided and healing had taken place alongside of areas where active lesions were present. In the animals in all experiments with the oil, regional lymph nodes were carefully examined for metastases and none were found. In the monkeys the glands never reached or invaded the external muscular coats of the stomach or intestine. Fibroplasia in the submucosa was abundant and always resulted in a layer of fibrous tissue between the growing glands and the muscle. The extremely short induction period for these lesions, varying between 48 and 213 days, also speaks against these lesions being neoplastic.

The pathogenesis of these lesions in the monkey cannot be reconstructed from this limited material. The pathogenesis was discussed in the case of the colonic lesions in the rat (9). Because of the similarity of these lesions to many human mucosal abnormalities an understanding of the mechanism of their production and fate would be useful in human pathology. An experiment is now in progress in which an attempt is being made to diagnose the presence of such lesions in the monkey before the animals become moribund so that the progression and fate of the lesions can be learned.

SUMMARY

Previous observations on the occurrence of infiltrating hyperplastic gastric mucosal lesions in rhesus monkeys who had ingested diesel motor lubricating oil are extended by additional examples of the disease. Similar lesions are produced in the colon by the same means. Two instances of similar but apparently naturally occurring gastric lesions in monkeys are reported. These lesions are described and their nature is discussed. Their similarity to human disease and, in several instances to human gastric and colonic carcinoma, are pointed out. These lesions are considered in the absence of incontrovertible proof not to be malignant neoplasms in spite of their morphological appearance.

REFERENCES

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