Gastric Absorption of 3,4-Benzpyrene

I. The Effect of Physiological Processes on Absorption

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Adenocarcinoma of the glandular region of the stomach has not been induced by feeding the fat-soluble carcinogenic hydrocarbons to mice and rats, although carcinoma of the forestomach and/or of the small intestine may occur (1, 4, 23, 28). It has been suggested that the glandular mucosa of the stomach is relatively resistant to tumor induction and that there may exist a "protective mucous barrier" which prevents contact between the carcinogen and the mucosal cells (2, 5, 11, 13, 15, 16, 28, 30). However, the glandular mucosa is susceptible to the action of carcinogenic agents when introduced directly into the wall of the stomach (12, 22, 29). In addition, gastric adenocarcinomas are reported to be induced by feeding heated lipids (6, 20, 21, 31), although similar experiments by others have failed (3, 15).

In a study of experimental gastric carcinogenesis, the gastric absorption of carcinogenic hydrocarbons requires investigation. The role of the stomach in digestion and the differences between gastric and intestinal absorption, as well as the various factors influencing these processes, have been largely neglected in the study of experimental gastric carcinogenesis.

It has been shown previously (24, 25) that 3,4-benzpyrene in certain vehicles penetrates the various coats of the stomach. Also, the fluorescent constituents of tobacco tar are absorbed in the gastrointestinal tract (27).

In the present study the absorption of 3,4-benzpyrene in normal mice and cats has been investigated in different parts of the gastrointestinal tract, taking into account the physiologic background of absorption of ingested material.

MATERIALS AND METHODS

The rate of absorption of 3,4-benzpyrene (BP) (Eastman Kodak Co., Rochester, N.Y.) in various solvents, after gastric instillation, was studied by determining the blue fluorescence of the material with the fluorescence microscope technic (Reichert Lux UV with a Philora HPW 125-watt mercury vapor lamp). The intensity and the penetration power of the blue fluorescence was estimated by using an arbitrary gradation (cf. Fig. 2). However, spectroscopic estimations were not made, and therefore it is unknown whether the blue fluorescence in the tissues depends on unchanged BP or on its blue fluorescent metabolites. Bearing this in mind, reference is made only to the absorption of BP in the present work.

About 600 healthy adult stock mice of both sexes, employed for several years in our studies, were used. Some experiments were conducted with C3H mice with similar results. Additional investigations were carried out with young cats, weighing 250—350gm. All the animals were kept under similar laboratory conditions, and the diet was sufficient and balanced, particularly with respect to vitamins A and B. No pathologic changes, including spontaneous tumors, were encountered. The animals fasted at least 12 hours before administration of BP, as well as during the entire experiment. Thus, the stomach was nearly empty, and dilution of the solutions instilled was prevented, except that due to the normal gastric secretion.

The amount of solutions administered by stomach tube was 0.1 cc. per mouse and 0.5 cc. per cat. The animals were killed by decapitation 5, 15, 30, or 60 minutes, or 24—48 hours after feeding. The organs, after fixation in 10 per cent neutral formalin solution (from 1 to 10 hours), were cut on the freezing microtome at 10 μ thickness, mounted in anhydrous glycerol, and examined (at least 5 hours after autopsy). Control observations were made on unfixed and unmounted specimens. Imersion of the organs in the fixative solution for less than about 10 hours produced no essential changes in the localization and in the intensity of the blue fluorescence in the tissues. Other specimens were imbedded in paraffin and stained in the usual manner for comparison.

Because carcinogenic hydrocarbons are fat-soluble, it seems likely that the mechanism of their...
absorption is linked to that of lipids in general. The following vehicles were used: (a) natural fats: olive oil and arachidis oil; (b) natural fats, emulsified in natural ox bile, with controls of (c) natural ox bile only; (d) lecithin, representing natural phosphatides. Parallel experiments were made using (e) unsaponifiable light petrolatum and (f) emulsions of light petrolatum stabilized with Aerosol OT, or with sodium taurocholate (17). In addition, a great number of substances (g), possessing simultaneously lipo- and hydrophilic properties, were examined, both in aqueous and in nonaqueous solution (8, 24, 25). The physico-chemical properties are discussed in the following report (7).

The BP concentration varied from 0.5 to 0.01 per cent, owing to the differences in the solubility of BP in various solvents. For adequate comparison, results have been corrected to standard conditions.

To determine the possible importance of individual variation in the amount of gastric secretion to the rate of absorption, histamine experiments were carried out: 0.2 cc. of histamine solution, containing 0.5 mg. of histamine hydrochloride prodorli, was injected subcutaneously in mice every 15 minutes during this experiment.

RESULTS

Stomach of the Mouse

Only the most important observations made on the blue fluorescence in the wall of the mouse stomach 80 minutes after gastric instillation of BP in various mediators are presented in the following (cf. Figs. 1, 2, for orientation).

Glandular stomach.—BP, dissolved in natural fats or in light petrolatum, did not cause any visible blue fluorescence in the glandular mucosa, either in the highest concentration (0.5 per cent) used, or when the animals were killed after 6 hours, during which time only a comparatively small amount of the fat solution had entered the intestine, and thus the surface of the glandular mucosa had in close contact with the BP solution all that time. These results agree with the observations made in feeding experiments which have failed to produce adenocarcinomas, and in which natural fats have been mainly employed as solvent for the hydrocarbon.

With all the other vehicles, signs of absorption of BP were observed in varying degrees. The intensity of fluorescence as well as the penetration power varied considerably in different groups, but the blue fluorescence was qualitatively similar in all cases.

BP, dissolved in natural fats (e.g., in arachidis oil) and emulsified in natural ox bile, caused a distinct blue fluorescence in the most superficial layer of the glandular mucosa. The same phenomenon was observed when lecithin was used.

Emulsions of light petrolatum are, in contrast to plain light petrolatum, able to penetrate the most superficial layer of the glandular mucosa. However, the blue fluorescence disappeared rapidly in these cases.

In general, the fluorescence intensity was greatest at the mucous surface, becoming gradually weaker deeper in the stomach wall (cf. Fig. 2). BP seemed to penetrate comparatively rapidly to a certain depth which is characteristic of each vehicle; after about 30 minutes the depth of penetration increased only insignificantly, indicating that further transport of the fat-soluble BP is slow. The depth of the penetration did not depend on the actual thickness of the glandular mucosa; in regions in which the thickness of the wall was less, all layers of it had taken up blue fluorescent material, while in the thicker regions of the same stomach only the equal superficial layers were fluorescent. In several cases the wall of the pyloric region was thinner than that of the others. In cases in which the whole mucosa had absorbed BP, there appeared to be a well developed network in the muscle layers (24). A similar structure existed in the corresponding part of the forestomach as well (Figs. 3 and 4). There were great differences in penetration power among various solvents (25).

The nuclei were free of fluorescent material. There were no noticeable differences in the intracytoplasmic distribution and localization of BP in various cell types; intracytoplasmic fluorescent granules were not noted (Fig. 5).

A strong mucigenic action, most probably of a local nature, was observed in connection with sodium cholate, sodium taurocholate, and sodium myristyl sulfate. Some of the earlier authors have explained that the limited gastric absorption may partly depend upon the so-called washing effect of the gastric secretion. However, a strong secretion, induced artificially by histamine administration, weakened the penetration power of the vehicles only slightly.

When BP, solubilized in aqueous solution, was given as a single application, the blue fluorescence disappeared from the glandular mucosa in about 30–45 minutes; but when given in anhydrous lipohydrophilic substances with a strong penetration ability (e.g., polyethylene glycols, Tritons), the time of disappearance was 6 hours.

Forestomach.—BP, dissolved in any of the vehicles studied, easily penetrated the wall of the mouse forestomach (Figs. 2, A–D, 3, and 4), as
previously described (24). Similar observations were made on the esophagus and the ridge between the forestomach and the glandular stomach (Fig. 4), both of which are also lined with squamous epithelium. In general, the blue fluorescence disappeared from the forestomach wall, in 24–48 hours. All the results are compatible with the observations made in feeding experiments in which tumors developed only in the forestomach (1, 4, 23, 28).

**STOMACH OF THE CAT**

Experiments were made on cats, because the cat's stomach rather closely resembles the human stomach both anatomically and physiologically.

The results were essentially similar to those obtained in experiments with mice (Figs. 6 and 7).

**INTESTINE OF MICE AND CATS**

Absorption of the blue fluorescent material from the lumen of the intestine was, in general, similar in mice and cats. BP dissolved in various fats, emulsions, and in the simultaneously lipoid and hydrophilic substances easily penetrated the tissues of this segment of the alimentary canal. A very strong BP fluorescence was seen intracytoplasmically in the structures of the wall of the small intestine (Figs. 8 and 9) and in that of the large intestine.

Differences in penetration power among the various types of vehicles for BP corresponded to well known physiologic principles.

**THORACIC DUCT OF MICE AND CATS**

After instillation of BP into the gastrointestinal tract, blue fluorescent material was seen in the contents of the mesenterial lymphatics, and in lymph of the thoracic duct in connection with all types of vehicles used.

In addition, we have reported in another connection that at least a considerable part of instilled BP is carried further by the chylomicrons of the blood (26). As is known, the water-insoluble alimentary fats in the blood are transported as chylomicrons, the composition of which varies depending on several factors (9).

**DISCUSSION**

Gastric absorption was studied earlier chiefly by ligating the pylorus and, after a variable period, by withdrawing samples of stomach contents and of blood. However, in experiments on mice, the histologic technic may be the most suitable one, because operative procedures change the physiologic conditions in the stomach. In addition, the histologic method has the advantage of following the absorption directly in the stomach wall, which is the actual problem. The word "absorption" has here been used in its broadest sense, including passive penetration. Although the limits of the sensitivity of fluorescence microscopy as used in the present work are not fully known, it is certain that this technic is considerably more accurate than those previously used.

As recorded in Fig. 2 D, BP dissolved in plain fats or in light petrolatum did not cause any visible blue fluorescence in the glandular mucosa. This is understandable, because little or no fat is absorbed by the stomach wall under normal conditions (cf. 5, 14, 19).

First, after being emptied into the small intestine, alimentary fat comes in contact with the bile, one of the most important factors in fat absorption. The sodium salts of the bile acids are able to make fat-soluble substances (18, 92), including carcinogenic hydrocarbons (8), water-soluble. According to the lipolytic theory of fat absorption, fat, before absorption, is enzymatically hydrolyzed into split products, which are watersoluble. However, according to the fat partition theory (10), a certain amount of fat, associated with bile salts and some hydrolytic products, is absorbed directly in insoluble form; the size of the fat particles must be less than about 0.5 µ (cf. 9).

However, conditions in the intestine and in the unchanged stomach are not comparable—practically no emulsifying or lipolytic agents occur in normal stomachs. Under certain pathologic conditions, e.g., hypochlorhydria or achlorhydria, gastric absorption of fat-soluble substances would, however, be possible. Though it is unknown how often regurgitation of the contents of the duodenum into the stomach occurs in the so-called precarcinogenic period of gastric carcinogenesis, it has been considered a possible contributory agent in the genesis of gastric cancer. In addition, Ivy (18) observed that the pyloric mucosa and sometimes the mucosa along the lesser curvature—i.e., in those regions in which about two-thirds of gastric carcinomas occur—was stained yellow with bile pigment when the contents of the duodenum were present in the stomach.

It appeared in the present work that BP, dissolved in fats and emulsified in natural bile, as well as solubilized by some bile salts or in synthetic bile, caused a distinct penetration into the superficial layer of the wall of the glandular stomach. Furthermore, BP, solubilized both in aqueous and in nonaqueous synthetic vehicles, possessing simultaneously lipoid- and hydrophilic properties (cf. 24, 25), is easily absorbed by the glandular mucosa of the normal stomach in mice and cats. All the substances mentioned above have a
common characteristic: they are able to bring the fat-soluble carcinogenic hydrocarbons into aqueous solution. The physico-chemical properties of the vehicles used are reported in the following paper (7). There is no real basis for the postulated “mucous barrier” to absorption of carcinogenic hydrocarbons in the stomach.

It is not sufficient that the carcinogens are absorbed by the stomach wall. The agents must remain in situ for some time before tumors develop (cf. 11). However, it was noted in the present investigation that the blue fluorescence of the ingested material disappeared from the normal glandular mucosa during a time which evidently is too short to induce adenocarcinomas of the stomach. It seems plausible, however, that gastric adenocarcinoma could develop in a stomach which is pathologically altered, since this would provide an entry for some fat-soluble carcinogens (e.g., “heated lipids”). Thus, though the significance of chronic atrophic gastritis in gastric carcinogenesis has not been established with certainty, it may be said that a great bulk of gastric carcinomas in man arises from gastritis. Because the intestinal wall, physiologically, and the coat of the normal stomach in the presence of certain solvents absorb the fat-soluble carcinogen (cf. Figs. 5, 6, and 8), it can be expected a priori that the altered, newly formed coat of the gastritic stomach will also resemble the actual intestinal lining when factors participating in the absorption of fat-soluble substances are present. In addition, it is understandable that owing to the alterations in the structure of the wall of the gastritic stomach (together with the general weakness of the lacteal system in it) the carcinogen could not be further transported as easily as it could following absorption by the intestine. It may be that gastric adenocarcinoma occurs in a stomach in which there is a “functional and anatomic intestinalization.” Certain clinical observations strongly support this assumption.

Investigations, planned along the principles presented above, are now in progress.

**SUMMARY**

Gastric absorption of 3,4-benzpyrene in mice and cats was investigated following gastric instillation of a single dose of the hydrocarbon, taking into account the physiologic knowledge of absorption of ingested material in different parts of the gastrointestinal tract. The rate of absorption was determined directly in the wall of the normal stomach with fluorescence microscopy.

No signs of absorption by the gastric glandular mucosa were observed after administration of the hydrocarbon in natural fats or in liquid petrolatum. These results are in agreement with the observations made in feeding experiments in which adenocarcinomas were not produced when fats were used as solvents for the carcinogen.

On the contrary, benzpyrene was able to penetrate into the glandular mucosa of the stomach when dissolved in fats or in light petrolatum and emulsified in natural bile or solubilized by some bile constituents (both natural and synthetic ones).

A strong absorption occurs when synthetic substances possessing simultaneously certain lipoid- and hydrophilic characteristics, with the aid of which carcinogenic hydrocarbons are brought into aqueous solutions, are used as vehicles for the hydrocarbon. It is of great significance that these substances have certain common properties with bile. However, the blue fluorescence disappears comparatively rapidly from the glandular mucosa of the normal stomach.

The results have been discussed with particular reference to some physiologic facts and pathologic alterations in the stomach.

**REFERENCES**

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