Diet and Epithelial Hyperplasia in the Forestomach of Rats and Mice

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Since Singer (19) in 1913 observed hyperplasia and ulceration of the forestomach epithelium in rats fed bread and wood shavings, there have been numerous studies to determine the etiology of the lesions. Papenheimer and Larimore (14) showed that a faulty diet would produce the changes, but there is still divergence of opinion as to the nature of the deficiency and the mechanism of lesion formation.

While benign changes have been described by most authors, Fibiger (6) reported that squamous carcinoma of the forestomach epithelium was produced in rats and mice by feeding cockroaches infected with Gongylonema neoplasticum (Spiroptera neoplastica). He believed the evidence was conclusive that Spiroptera could serve as the etiologic agent of both benign and malignant changes. Carcinoma was thought to be a more advanced stage of the benign hyperplasia. Studies performed since the discovery of accessory food factors have shown that Fibiger's diet was nutritionally poor and that even without the worms it would induce some alterations (15). With an improved diet, Passey, Leese, and Knox (15) were able to increase the life span of infected animals without the production of extensive changes. Cramer (4) accepted Fibiger's interpretation of carcinoma in a few cases but made the reservation that something more than Spiroptera and poor diet was necessary to produce it.

Hoelzel and Da Costa (11) found ulcers in the epithelium of both the forestomach and the glandular portion in rats and mice when low protein diets were fed and also when the animals were fasted every second day. Since gastric stasis resulted from either method, they suggested that irritation from unbuffered acid produced the changes. Some support for this hypothesis was provided when Matzner and his associates (13) demonstrated that pepsin and hydrochloric acid could induce ulceropapillomas. These investigators observed that more extensive lesions developed when pepsin and hydrochloric acid were fed together than when either was given alone.

Irritation either by parasites or gastric juice is the only explanation that has been advanced for the mechanism of lesion formation, though without attempting to elucidate this mechanism numerous reports have suggested that the changes are a result of vitamin deficiency. Thus stomach lesions were found in rats suffering from vitamin B1 deficiency by Dalldorf and Kellogg (5) and Sure and Thatcher (20), while Findlay (7) obtained them only with vitamin B2 deficiency. These studies were made, however before the synthetic vitamins became available and other deficiencies also may have been present. Howes and Vivier (12) demonstrated that lesions occurred despite adequate protein intake if there was a vitamin B deficiency, and concluded that the whole vitamin B complex is necessary to maintain normal forestomach epithelium. Although Wobbach and Howe (22) proved that vitamin A is essential for the maintenance of normal epithelium in general, they did not find forestomach changes in vitamin A deficient animals. On the other hand, extensive lesions at this site were described by Fujimaki (9), and attributed to vitamin A deficiency, but further study (10) showed that undetermined factors were also important in their production. From a recent report of well controlled studies started by Fibiger (8) we learn that vitamin A deficiency under favorable conditions will increase the incidence of lesions, but that additional unknown factors are necessary completely to prevent their formation. While they offer no explanation of the mechanism of formation, Brunschwig and Rasmussen (1) suggest that when a certain degree of malnutrition exists in the rat, such malnutrition not necessarily being synonymous with absolute loss of weight nor with the lack of any specific type of food or vitamin, the forestomach mucosa reacts by localized foci of cellular proliferation.

Hyperplasia and ulceration are the normal reactions of the forestomach epithelium to mechanical or chemical irritation. Bullock and Rohdenburg (2) inserted into the forestomach by gastrotomy either a celluloid ball covered with pin points or pieces of cork with protruding pig bristles, and observed extensive changes, similar in many respects to those obtained by Fibiger with parasites. Chemical irritation with a piece of rubber sponge impregnated with Schallach R, or pine tar oil introduced in a similar manner,
also stimulated epithelial hyperplasia in this region. Irritation from ingested hair was thought to be an etiologic agent in the bread flour regime of Pappenheimer and Larimore (14). However, the inclusion of 10 per cent chopped hair in a sufficient diet rarely produced changes, and although hair is normally ingested by rats in small amounts, forestomach changes are not found when an adequate diet is allowed. If hair is an etiologic factor it must be assumed, therefore, that the epithelium has been made abnormally sensitive by an incomplete diet.

By using two types of diet with rats of a single colony, Sharpless (17, 18) demonstrated that the lesions can be caused by more than one deficiency. A deficiency of protein in one regimen and a deficiency of the vitamin B complex in the other produced the changes. Adequate controls made it possible to correct the deficiency and get approximately normal stomachs without changing the other nutritive properties of the diet, and without affecting the growth of the rat. Recently Chen (3) found that agar-agar and glucose provided considerable protection in starved rats and questioned the theory that nutritional deficiency is important in the etiology of the lesions.

In all these studies, the lesions are believed to have been due directly to a dietary deficiency, to the action of local factors alone, or to local factors acting on epithelium with lowered resistance to irritation. The local factors that have been proposed are mechanical irritants such as hair, parasites, and hard particles in the food, as well as chemical irritants such as pepsin and hydrochloric acid. Bile that may have been regurgitated into the stomach is a second possible chemical irritant, although it has not been discussed in the literature in connection with changes in the rat's stomach. The divergence of reported results would probably not great, if all the facts were known. However, some of them may be susceptible of interpretations different from those suggested by the authors. Using the two diets (17, 18) previously described, we have performed additional experiments to obtain data for an explanation of the lesions.

METHODS

In all experiments young rats, males and females between 55 and 80 gm. in weight, were used, and litter mate controls were fed the basal ration. Two types of diet were studied. One, the white flour diet, was composed of approximately 90 per cent white flour; the other, the low protein diet, was made up of purified foods low in protein. The experimental period was 42 days for the former and 90 to 100 days for the latter. Food and water were given ad libitum.

At the end of the experimental period all animals were killed with chloroform and their stomachs examined immediately. If one or more typical changes were found in the gross examination, the animal was recorded as having lesions. Except where the variation was great, no attempt was made to determine the protective power of the diet by differences in severity of the alterations.

RESULTS

The changes in the forestomach epithelium consist of well circumscribed ulceropapillomas that are usually round or oval in shape, although in some animals they blend and cover one-half or more of the surface. Sometimes the ulcerated portion is a narrow, irregularly elongated fissure, 5 mm. or more in length, surrounded by the typical thick epithelium. The border between the forestomach and the glandular portion is a slightly elevated ridge of squamous epithelium, called the limiting ridge. It is usually smooth, but in these animals it is more prominent and thickened irregularly in the form of beads. It appears to be one of the first areas affected, and may be prominent and beaded when no macroscopic ulceropapillomas are present.

The effectiveness of protective factors was determined in this study by the macroscopic appearance of the forestomach epithelium when the animal was killed. The changes were recorded as slight or mild when one to five small individual ulceropapillomas or beading of the limiting ridge were present. Severe or extensive changes were recorded when one-half or more of the surface was covered with ulceropapillomas.

Although sections were made of representative stomachs in all the groups studied, a detailed account of the microscopic alterations has not been included because they were essentially the same as those that have already been described so frequently.

Experiment 1.—The low protein diet previously described (17) had the following percentage composition: casein 4.0, dried yeast 5.0, salt mixture 4.0, butterfat 5.0, cornstarch 8.2. When 0.2 per cent cystine was added the stomach remained normal and growth was increased from 2 or 3 gm. to approximately 15 gm. per week. Protection under these conditions could have been provided either by cystine or by alleviation of chronic starvation. In order to eliminate the variation in intake, the food of 6 rats fed the cystine-supplemented diet was limited to that eaten by litter mate controls of the same weight fed the basal regimen. Two of the 6 fed cystine had one small lesion each, whereas the forestomach of each control was completely covered with lesions, a demonstration that sulfur-containing amino acid deficiency, and not starvation, was the main etiologic factor in the low protein diet.
**Experiment 2.**—Preliminary experiments showed that 12 per cent casein in the low protein diet described above is the minimum amount that will prevent the lesions. In order to test the theory that pepsin and hydrochloric acid will produce the change, 5.0 per cent scale pepsin was fed together with 12 per cent casein, and 0.05 N hydrochloric acid was given for drinking water. This diet was used in order to provide protection with as nearly as possible the same ration and conditions that produce the changes spontaneously. Of 9 rats given this diet 5 had one or more areas of typical ulceropapillomas, whereas their controls had normal stomachs, but in none of the 5 were the lesions so extensive as those caused by the low protein diet alone. This experiment demonstrated that although pepsin and hydrochloric acid could bring about changes, they alone were not responsible for the extensive lesions following the low protein regime.

**Experiment 3.**—A summary of some long continued feeding studies with the low protein diet is included under this experiment. Of 168 rats thus fed for more than 10 months, 3 have developed a squamous carcinoma in the mouth and one in the vagina. More than 1,800 animals of the same colony have had the normal stock ration for a similar period and no squamous carcinoma has occurred. Of 24 mice (C57 black strain) given the same diet for more than a year, 2 developed epithelial cysts on the tongue similar to those found in the stomach. This summary shows that with the low protein diet changes occur occasionally in areas of squamous epithelium other than the forestomach. The direct cause of these changes was probably some factor other than the diet, but in view of the experience with stock animals the regimen must have predisposed the rats to the action of this unknown factor.

**Experiment 4.**—The white flour diet had the following percentage composition: white flour 90.0, butterfat 5.0, calcium lactate 2.0, sodium chloride 2.0, and ferric citrate 0.1. The defect is a biologically poor protein and a deficiency of the vitamin B complex. The purpose of this study was to determine if a correction of the protein defect would prevent the changes. Eight rats were given the diet supplemented with 15 per cent casein, an amount known completely to prevent changes when the vitamins are adequate. Three of the 8 had stomach changes. Other experiments in which gelatin was added, or in which the supplements were pure amino acids, demonstrated conclusively that biologically poor protein was not the only cause of the lesions in rats fed white flour.

**Experiment 5.**—Previous studies have shown that practically normal forestomach epithelium can be maintained in the white flour diet with supplements of riboflavin, cystine, nicotinic acid, and a water-soluble concentrate of rice polishings (18). With pyridoxin and thiamin substituted for the rice polish concentrate, 31 of 49 rats had lesions. When choline was also fed only 8 of 58 animals had stomach changes. However, all 6 rats fed the white flour diet supplemented with choline alone had extensive stomach changes. Approximately 90 per cent of the animals fed the unsupplemented ration had abnormal forestomach epithelium. In none of these experiments did the supplements significantly improve the food consumption or growth.

**Experiment 6.**—In the studies mentioned above—except in the animals fed choline—intestinal contents were often found in the stomach. Choline caused a slight diarrhea and probably helped to prevent regurgitation of intestinal contents into the stomach. Since it is well known that bile is irritating, 0.5 per cent sodium taurocholate was incorporated in the supplemented white flour regimen that gave the most complete protection. All 14 rats fed this diet had extensive changes in the forestomach epithelium while only 3 of 9 litter mate controls were affected, and each of these 3 had but one small abnormal area. In order to determine whether this was due to the action of sodium taurocholate alone or to the combined action of a poor diet and the bile salt, 9 rats were fed the normal stock ration supplemented by 0.5 per cent sodium taurocholate. Despite the fact that these animals ate 40 per cent more food, and therefore more sodium taurocholate, than those fed white flour, all had normal stomachs. Since the concentration of sodium taurocholate in contact with the stomach epithelium was probably similar in the two experiments, the stock diet must have made the epithelium less sensitive to irritants. As a whole these experiments show that in those diets where it may be regurgitated into the stomach, bile should be considered one of the etiologic agents of the stomach lesions only if the resistance of the epithelium has been impaired by a poor regimen.

**Experiment 7.**—In order to demonstrate further that the lesions in rats fed the white flour diet were not caused by chronic starvation, the food intake of 10 rats fed the normal stock ration was regulated so that their weight was kept constant. When examined at the end of the 6 weeks, the stomachs of all were normal. Of 9 litter mates fed the unsupplemented white flour diet, 8 had stomach changes. This leads to the conclusion that chronic starvation of the type that occurs with these deficient regimens does not allow sufficient contact between the epithelium and the local factors to produce the lesions.

**DISCUSSION**

If the lesions are the direct result of a dietary deficiency, areas of squamous epithelium in other parts
of the body should also present hyperplasia and ulceration, but despite the presence of extensive changes in the stomach the squamous epithelium of the renal pelvis, vagina, and mouth are usually normal. Furthermore, if stomach lesions are produced by a specific inadequacy, this should always have the same effect. Some authors have referred stomach changes to a lack of protein, vitamin A, B₁, or B₂, yet with the same diets others have obtained no changes. On the basis of these observations it must be concluded that the lesions are not produced directly by incomplete regime.

Bullock and Rohdenburg (2) demonstrated that hyperplasia is the normal reaction of the stomach to either mechanical or chemical irritation. However, it has been shown that hair, pepsin and hydrochloric acid, and bile, the irritants that can come in contact with the forestomach epithelium, are not capable of inducing epithelial changes when a good diet is fed. Experiment 6 indicates that a bile salt, sodium taurocholate, effected changes when the diet was poor. In experiment 2, pepsin and hydrochloric acid induced some changes in the stomachs of rats fed a suboptimum regimen. Together with reports in the literature on the effect of ingested hair, this evidence suggests that an incomplete diet makes the epithelium sensitive to irritants that normally do not affect it. That the forestomach mucosa may not have normal resistance to proliferative stimulation may also be deduced from the observation that changes occur occasionally in other areas of squamous epithelium.

In the long continued studies described in experiment 3, there were 4 cases of squamous carcinoma in areas other than the forestomach. Two mice developed epithelial cysts on the tongue that were similar to those found in the forestomach. Pyridoxin, riboflavin, and nicotinic acid are all known to be essential for the maintenance of normal squamous epithelium. Although in the 6 week experimental period dermatitis was not obtained in their absence, it must be assumed that a subnormal amount of the vitamins was present and that the epithelium had abnormal resistance to irritants.

One possible explanation of the mechanism for the formation of lesions was provided by the work of Hamnett (see Reimann, 16). Hamnett's theory is that --SH is the essential stimulus for cell proliferation and that the strength of the required stimulus depends upon the amount of inhibitor (oxidized sulfhydryl) present. Furthermore, Reimann believes that either physical or chemical irritation will free --SH locally. (If the concentration of the inhibitor were reduced, less irritation should be required for proliferation.) A deficiency of metabolizable sulfur such as that provided by the low protein diet, should give the necessary conditions for producing proliferation with less than normal stimulus. Although unassailable proof of this explanation must await further study, some similar chain of reactions is indicated by the experimental results.

In addition to lowering the resistance of epithelium to irritation, dietary deficiencies also provide an opportunity for prolonged contact between the local chemical irritants and the epithelium. Thus absolute starvation allows constant contact between the epithelium and the gastric juice. Subnormal insulation of the mucosa from local factors, and dilution of the gastric juice, would also be expected in partial starvation. Some bile probably enters the stomach during complete starvation because there is no material present to stimulate normal intestinal movement or to act as a physical barrier. Ingestion of some food or inert material should erect a physical barrier to this abnormal movement of bile unless the muscle tone of the intestinal tract were impaired or abnormal peristalsis occurred. Deficiencies of thiamin, riboflavin, and possibly some other members of the B complex impair muscle tone. Choline increases muscle tone of the intestinal tract (21). In the white flour diet alone it had no effect but with other members of the B complex it was an important factor in the prevention of lesions (experiment 6).

Regardless of nutritive value, it is clear from the above discussion that any substance that will either prevent bile from getting into the stomach or will insulate the epithelium from contact with the local factors should help to prevent changes in the forestomach. Agar-agar would serve as a physical obstruction to the regurgitation of bile, stimulate normal intestinal movement, and dilute as well as help to eliminate hydrochloric acid and pepsin. Chen's (3) observation of the protective action of glucose and agar-agar might be explained on this basis.

As a whole the experiments show that a large number of dietary constituents are concerned in maintaining normal conditions in the gastrointestinal tract. The action of these constituents is interdependent, so that a deficiency of one will prevent effective action by the others. In the dietary treatment of gastrointestinal derangements, therefore, better average results should be expected from therapy with the whole vitamin B complex together with biologically good protein than would be possible from large doses of one substance alone.

**SUMMARY**

Under the conditions described a deficiency of cysteine, riboflavin, pyridoxin, or choline induced hyperplasia and ulceration of the forestomach epithelium in rats. Sodium taurocholate or pepsin and hydrochloric...
acid increased the incidence of lesions in animals fed the poor diets, but caused no macroscopic changes in rats fed the stock ration. Neither did the limitation of stock diet intake cause any visible lesions in the forestomach, and the body weight remained constant in young rats.

The action of the protective factors is interdependent, so that a deficiency of one may prevent effective action by others. From these facts and the observation that squamous epithelial changes were occasionally observed in areas other than the stomach, it is postulated that the mechanism of formation of the gastric lesions is the irritation of abnormally sensitive epithelium by hair, hard food particles, pepsin and hydrochloric acid, or bile.

REFERENCES

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