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# Effects of Diet and Stress on Gastric Lesions in Albino Rats and Guinea Pigs

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# EFFECTS OF DIET AND STRESS ON GASTRIC LESIONS IN ALBINO RATS AND GUINEA PIGS

by

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A Thesis in Partial Fulfillment

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Master of Science in the Field of Nutrition

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Each person whose signature appears below certifies that he or she has read this thesis and that in his or her opinion it is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# INTRODUCTION

Peptic ulcer remains one of the major diseases of the civilized world, affecting approximately 5 to 10 per cent of the adult male population in the United States and England (Ivy, 1950; Barborka, 1955; Moeller, 1964), and claiming approximately 12,500 lives in the U.S. alone in one year (Blumenthal, 1968). Not only does this disease affect the health of our nation but it causes tremendous economic losses, e.g. the cost to the United States is approximately one billion dollars a year. Although study has been given to the management of peptic ulcer in the last century, and many etiologic factors are recognized, the basic cause is still unknown.

Some investigators (Ivy, 1950; Dragstedt, 1959; Furguson, 1962) postulate that in patients with ulcers there is a local decrease in the resistance of an area of the mucosa which precedes the digestion of that tissue. Perkel (1968) and Shull (1958) indicate that peptic ulcer is the end result of an abnormal physiology characterized by the inability of localized areas of the stomach and duodenum to resist the digestive action of gastric juice.

According to Code, et al. (1963) and Davenport (1968) the normal mucosa is part of a barrier composed of at least two components which resist the tendency of $H^+$ to diffuse from the lumen into the mucosa and of $Na^+$ to diffuse in the other direction. The exact mechanism of how this double barrier works is not known but it is believed that one layer consists of lipoprotein and that it forms the plasma membrane of the mucosal cells while the other may be composed of the mucous layer at the tips of the epithelial cells. This two component barrier

apparently serves as a protecting force. The mucus is not in itself a barrier to diffusion, instead, the real barrier is the membrane of the cells of the mucosa. The lipoprotein membranes allow only restricted movement of small ions through their water-filled pores, but they are rapidly penetrated by fat soluble compounds of any size. If the penetrating compound is toxic, it disrupts the barrier and increases its permeability to ions. For example, 100 mN acetic acid, because it is fat soluble diffuses through the gastric mucosa seven times faster than the hydrogen ions of 100 mN HCl. This results in a breaking down of the barrier; consequently, hydrogen ions of the gastric contents move through it more easily exacerbating the damage. If damage is severe enough bleeding follows (Davenport, 1966).

Diet has played an important part in the therapeutic regimen for patients with peptic ulcer but it has been a controversial issue for many years. In the first century A.D. Celsus prescribed a smooth diet, free of acrid foods at a time when little was known about diseases of the stomach, and, as early as the seventh century A.D. practitioners prescribed milk for patients whose digestive symptoms suggested the possibility of peptic ulcer (Rosenak, 1951). Today, centuries later, the question of diet therapy still remains a paradox and one can see the importance of continued investigation along this line so that in due time ample clarity on this subject can be achieved.

Some work has been done and is being done with cabbage (Cheney, 1940,1944,1948,1949,1950,1952,1954,1956; Vargha, 1963), olives (Komarov, 1940; Apperly, 1943; Schneider, 1958; Nesterova, 1963; Moeller, 1964; Vermel, et al. 1966), and spices (Kellogg, 1929; Sanchez-Palomera, 1951; Schneider, 1956), in the hope of finding out if they engendered

preventive, curative, or causative effects in relationship with peptic ulcer.

For centuries spices have been used widely all around the world. At present one can hardly find a cupboard devoid of spices, and even though they are used in small amounts they play an important part in the dietary habits of the human race. One of the problems in diet therapy in peptic ulcer disease is that of tastelessness of the diet to one accustomed to these agents with resultant lack of patient cooperation. These diets are the results of physicians' warning for patients to avoid "spices and highly seasoned foods" without adequate evidence to support the effect of different spices on the gastric mucosa and the healing time of peptic ulcer.

It was the purpose of this study to (1) investigate whether any protective factor is present in fresh water-cured olives or fresh cabbage as compared with the heated supplements, against stress-induced gastric lesions in rats maintained on a nutritionally adequate diet for six weeks, (2) determine the relative amount of protective factor, if any, in water cured olives as compared with cabbage, (3) study the effect of black pepper in combination with fresh cabbage and olives and heated cabbage and olives in rats that were fed the same nutritionally adequate diet, and (4) investigate the effects of the same dietary components--cabbage and olives--on histamine-induced gastric lesions in guinea pigs fed heated lab chow for five weeks.

## REVIEW OF THE LITERATURE

Cabbage factor complex for peptic ulcers. Cheney and other investigators (Cheney, 1944, 1949; Gianelli, 1945; Vargha and Damrau, 1963) demonstrated that a dietary factor found in cabbage plays an important role in the prevention and therapy of peptic ulcer. In a series of patients with peptic ulcer, treatment with standardized cabbage factor (or Vitamin U) found only in the fresh juice afforded prompt relief of pain and hyperacidity with eventual healing of the ulcer niche in the great majority of cases (Vargha and Damrau, 1963). The fresh cabbage juice loses the anti-ulcer factor even at room temperature (Vargha and Damrau, 1963).

When the authors compared their findings with a collection of cases recorded in the literature for other treatments, it was found that cabbage juice provided a 3 to 1 increased ulcer healing rate. Several hundred soldiers with ulcers were successfully treated with cabbage juice during the period of World War II. The cabbage factor also inhibited the development of cinchophen-induced gastric and duodenal ulcers in dogs, chicks, guinea pigs (Cheney, 1940,1942,1944,1948, 1950,1952,1954,1956) and rats (Vermel, 1966). Vermel suggested that fresh cabbage juice contains the Vitamin U factor which evidently includes in its composition "methylmethioninesulfonium."

Anti-peptic ulcer factor and olive oil. Very little investigation has been done with olive oil to determine its effect on gastric secretion. Komarov (1940) believed that unsaturated fats like olive oil, when given in small amounts are more effective than saturated ones

in easing the distress of peptic ulcer patients. In Moscow, Nesterova (1963) enriched "anti-ulcerous diets" with olive oil and found favorable effects on the healing of peptic ulcers. Schneider (1956) claims that large amounts of oil must be used in order for the gastric secretion to be inhibited. Studies done with dogs having vagally innervated gastric pouches showed that significant inhibition of gastric acid output occurred when olive oil was administered in different doses at different times (Long and Brooks, 1965).

Spice and peptic ulcer. Knowledge about the action of spices on the gastrointestinal tract is scanty (Farrell, 1928; Kellogg, 1929; Sanchez-Palomera, 1951) and the references found in the current textbooks of physiology and pharmacology (Howell, 1946; Wright, 1947) are vague and not documented. Reports from different investigators seem to conflict regarding the effect of spices on the stomach ulcers. Some claim destructive effects; others say the opposite.

Studies have been undertaken by Schneider (1956) to evaluate the effect of different types of spices, in the amounts commonly used, upon stomach and peptic ulcer. He maintained fifty patients with peptic ulcer on an ulcerogenic regime where they were given either cinnamon, nutmeg, allspice, mace, thyme, sage, paprika, caraway seed, chili pepper, cloves, black pepper, or mustard seed in doses of .05 to .9 g three times daily with meals for periods up to 180 days. Only five patients in this group developed adverse symptoms during the administration of the spices; two with black pepper, one with chili pepper, one with nutmeg, and one with mustard seed. The patients who received black pepper developed severe burning epigastric pain, belching, and

nausea within 24 hours, and required discontinuation of the spice. Severe hyperemia and edema of the gastric mucosa were also induced by the black pepper.

Stress-induced peptic ulcer in laboratory animals. There are a number of methods used to produce experimental peptic ulcers; however, the importance of various factors in producing ulcers in man is still controversial (Brodie, 1968).

In the last ten years the restrained (immobilized) rat, or stress-induced ulcer preparation has been widely used (Guth, 1964). This type of ulcer appears to be the experimental counterpart of Curling's ulcer (Artz, 1966) or human "stress ulcer" (Grosz, 1967). The production of this type of "ulceration" in the rat was first demonstrated by Selye in 1936. Since then, however, investigators in France (Bonfils, 1960) and in the United States (Brodie, 1960) have corroborated the finding that restraint produced mucosal "ulceration" of the glandular portion of the stomach.

Food deprivation and immobilization stress isolation have been successfully used separately and together to develop ulcers in rodents (Sines, 1962; Ader, 1964; Frisone, 1965; Essman, 1966). Animals with empty stomachs have been shown to have a correspondingly greater incidence of gastric lesions than those with food in the stomach (Essman, 1966). Ader (1965) indicated that ulcer development from immobilization stress in rats occurred with relatively equal frequency in group-housed and individually housed animals which were not handled or otherwise treated.

Recent preliminary studies by Baldwin and Baldwin (unpublished)

have indicated a higher incidence of gastric lesions in rats fed spice-containing diets.

## METHODS AND MATERIALS

### RAT STUDY

Animals. One hundred and twenty-eight female, 21-day-old Fischer albino rats weighing 27-32 g were purchased from Simonsen Laboratories, Inc., Gilroy, California, and were used in Study I. The rats were housed in community cages in a room of constant temperature of 78° F and a relative humidity of 40-50 per cent and fed Purina chow for a three day equilibration period.

Each group received tap water ad lib. The food was given in Fisher porcelain food cups, and these conicles were placed inside jars to minimize food loss. Throughout the six weeks of the experiment the rats were pair-fed by groups. The food was a moist homogeneous mixture for all groups as is described under "Diets" in this same section. The animals were weighed by groups at weekly intervals and their weights recorded for comparative purposes later on in the study.

The study was done in two separate experiments, one week apart, under identical circumstances.

Diets. The diets were all composed of an 18 per cent casein diet and contained equivalent amounts of protein, fat, carbohydrates, vitamins and minerals (Table II). All the diets contained either olives or cabbage. Specially prepared water-cured fresh olives or fresh cabbage were used for one half of the diet groups while the other groups contained the same foods after heating. The casein diet was composed of the following constituents in grams per 100 g of ration: Vitamin free casein 18.0, complete vitamin mixture 2.0 (Table III),

U.S.P. salt mixture XIV 4.0, vegetable oil 5.0, cabbage or olives 36.2, and sucrose to make 100.0 g. Two of the olive groups and two of the cabbage groups received black pepper in the proportion of 2 per cent by weight in addition to the rest of their diets.

The food ingredients except the olives or cabbage were mixed and refrigerated in separate cartons for each group. The cabbage and olives were freshly prepared each day immediately before feeding. They were chopped with a food chopper in approximately 3 mm pieces. The casein diet for each group was then weighed and the appropriate weight of cabbage or olive was mixed with it until a homogeneous mixture was made. This mixture was fed to the animals in the same diet group sequence between 6 and 8 o'clock every evening.

Stomach lesions. At the end of six weeks on the diet two animals from each group were randomly selected each day and were deprived of food but not of water for a period of 24 hours. At the end of this period they were placed in quantitative stress cages (Figure 1) for an additional 24 hours at a stress volume of 2.25 ml per g body weight. The rats were isolated during the stress period and neither food nor water was given to them for that time. At the end of the stress period the stomach, esophagus, and duodenum were removed under deep ether anesthesia, carefully opened along the greater curvature, washed with lukewarm water and pinned to styrofoam blocks. They were afterwards viewed under the dissecting microscope at 2X for lesions, engorgement, or other pathological manifestations. The lesions were counted and the size of each one was measured in length and width by an optic micrometer. A photograph of each stomach was taken. The stomachs were then

placed in 10 per cent formalin solution for future histological study.

The stress cages were the same as those designed for similar studies in this laboratory (Baldwin and Baldwin, unpublished data). They are made of two sheets of 1/4" lucite 12" x 7". Holes 3 mm in diameter were drilled in a matrix in each sheet so that stainless steel rods of the same diameter could be used to hold the sheets together and form the top, bottom, and ends of the restraint space. This arrangement allowed for easy adjustment of the desired height, width and length.

A preliminary study was done with 64 rats of the same strain. They were fed a casein diet free of cabbage or olives for six weeks and stressed at different volumes. The chosen volume was 2.25 ml/g body weight at which 50-70 per cent of the animals developed lesions.

## GUINEA PIG STUDY

Thirty male Hartley albino guinea pigs of the Curd strain and weighing 200-235 g, from Curd's Caviary and Animal Supply, La Puente, California, were used in Study II. The animals were housed in separate raised screen-bottom cages, given water and Purina guinea pig chow *ad lib* for a three day equilibration period. They were then divided into four diet groups as follows: (1) heated guinea pig chow plus fresh olives, 9 animals; (2) heated chow plus heated olives, 9 animals; (3) heated chow plus fresh cabbage, 6 animals; (4) heated chow plus heated cabbage, 6 animals. They were permitted water to which vitamin C (50 mg/100 ml) was added *ad lib* and were pair-fed by groups.

The cabbage and olives were prepared in the same manner as they were for the rats in Study I and were combined with the ground, heated chow and given to the guinea pigs in the same diet group sequence between 6 and 8 o'clock in the evenings. The chow was heated in an oven at 80° C for 24 hours to destroy any heat labile protective factor (Cheney, 1949). The animals were individually weighed weekly.

At the end of five weeks the guinea pigs weighed 450-500 g and were fasted for sixteen hours. At the end of this period they were injected intraperitoneally with histamine acid phosphate (2.5 mg/Kg body weight) in aqueous solution (1 mg histamine acid phosphate per ml) (Eagleton and Watt, 1965). Six hours after injection the stomachs and duodena were removed and examined under the microscope for number and area of lesions as described in the rat study above. Photographs of typical guinea pig stomachs without and with lesions are shown in Figures 4 and 5.

TABLE I

COMPOSITION OF RAT DIETS

| Constituent | I % | II % | III % | IV % | V % | VI % | VII % | VIII % |
|---|---|---|---|---|---|---|---|---|
| Casein | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| Corn Oil | - | - | - | - | 5 | 5 | 5 | 5 |
| Powdered Sugar | 68.7 | 68.7 | 68.7 | 68.7 | 64.6 | 64.6 | 64.6 | 64.6 |
| Olives | 36.2 | 36.2 | 36.2 | 36.2 | - | - | - | - |
| Cabbage | - | - | - | - | 36.2 | 36.2 | 36.2 | 36.2 |
| Vitamin Mixture | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Salt Mixture | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Black Pepper | - | - | 2 | 2 | - | - | 2 | 2 |

TABLE II

DIET FOR EACH GROUP OF RATS

| Group | Diet |
|---|---|
| I | Casein Diet + Fresh Olives |
| II | Casein Diet + Heated Olives |
| III | Casein Diet + Heated Olives + Spice |
| IV | Casein Diet + Fresh Olives + Spice |
| V | Casein Diet + Fresh Cabbage |
| VI | Casein Diet + Heated Cabbage |
| VII | Casein Diet + Heated Cabbage + Spice |
| VIII | Casein Diet + Fresh Cabbage + Spice |

TABLE III

COMPOSITION OF COMPLETE VITAMIN MIX

| Ingredients | Amount | Per Cent of Total |
|---|---|---|
| Thiamine chloride, milligrams | 300.0 | 0.03 |
| Riboflavin, milligrams | 400.0 | 0.04 |
| Pyridoxine hydrochloride, milligrams | 300.0 | 0.03 |
| Calcium pantothenate, grams | 2.0 | 0.20 |
| Niacinamide, grams | 2.0 | 0.20 |
| i-Inositol, grams | 10.0 | 1.00 |
| p-Aminobenzoic acid, grams | 15.0 | 1.50 |
| Folic acid, milligrams | 25.0 | 0.0025 |
| Biotin, milligrams | 10.0 | 0.001 |
| Menadione, milligrams | 100.0 | 0.01 |
| Vitamin $B_{12}$, milligrams | 2.5 | 0.00025 |
| Vitamin A, units | 2000000.0 | |
| Vitamin D, units | 170000.0 | |
| Vitamin E, units | 1500.0 | |
| Choline chloride, grams | 100.0 | 10.00 |
| Alphacel, to make 1 kilogram | | |

TABLE IV

COMPOSITION OF GUINEA PIG DIETS

| Constituent | G R O U P S I % | II % | III % | IV % |
|---|---|---|---|---|
| Heated Chow | 72.0 | 72.0 | 72.0 | 72.0 |
| Heated Olives | -- | 28.0 | -- | -- |
| Fresh Olives | 28.0 | -- | -- | -- |
| Heated Cabbage | -- | -- | -- | 28.0 |
| Fresh Cabbage | -- | -- | 28.0 | -- |

TABLE V

DIET FOR EACH GROUP OF GUINEA PIGS

| Group | No. Animals in Group | Diet |
|---|---|---|
| I | 6 | Heated Chow + Fresh Olives |
| II | 6 | Heated Chow + Heated Olives |
| III | 9 | Heated Chow + Fresh Cabbage |
| IV | 9 | Heated Chow + Heated Cabbage |

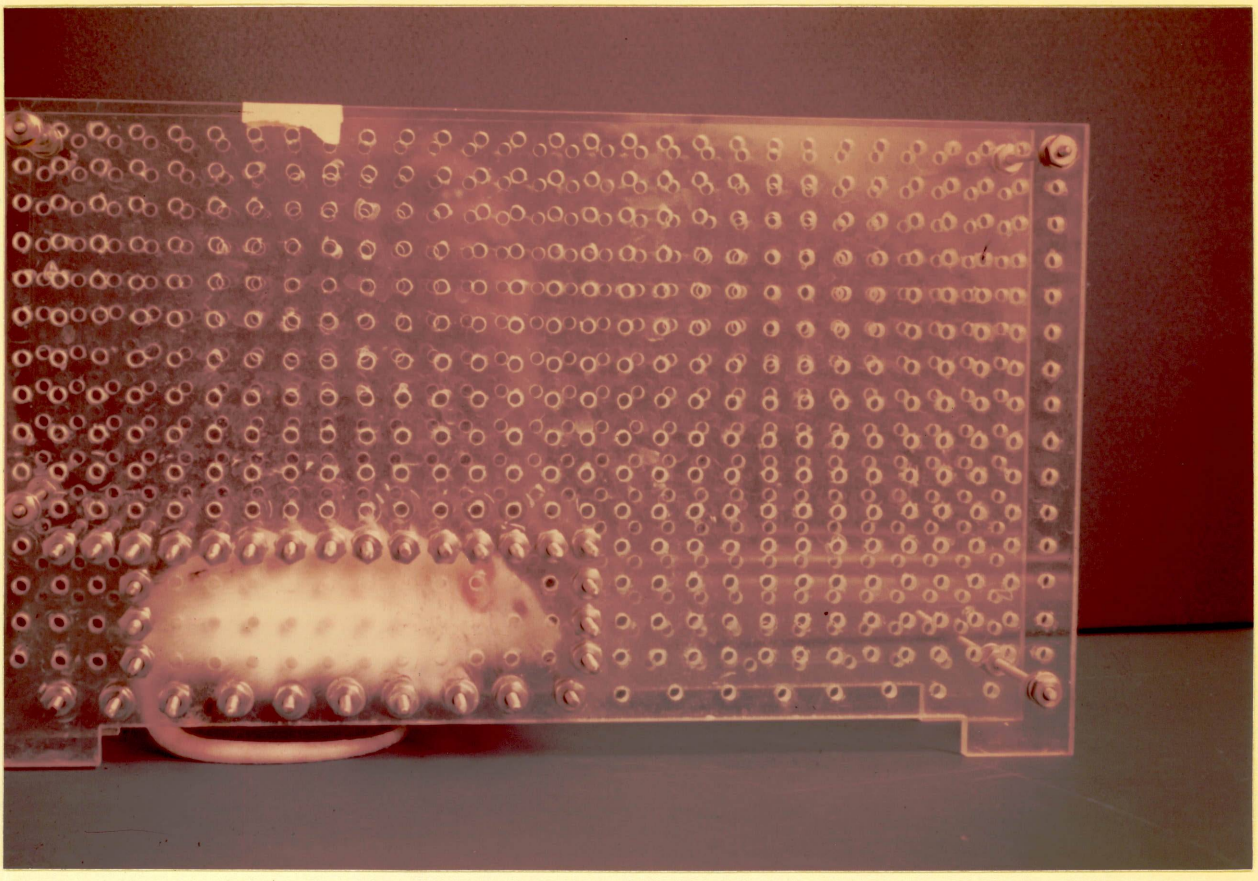

Figure 1. Quantitative Stress Cage Showing Rat Under Stress Confinement.

# RESULTS

## RAT STUDY

Throughout the six weeks on the diet marked differences in physical appearance between the animals that were fed spice and those that received no black pepper in the diet were observed. These differences first became noticeable after the animals in both experiments were on the experimental diet for eight days. The rats in groups 3, 4, 7, and 8 (the spice groups) showed signs of hair loss on the head and down the full length of the animals' backs and became considerably worse as they grew older, while groups 1, 2, 5, and 6 that were not fed spice had fluffy white coats of hair and sustained no losses of hair as is shown in the photograph on Figure 6. The larger rat was typical of the group fed the casein diet plus cabbage or olives while the smaller animal displaying the hair loss was typical of the group fed the nutritionally equivalent diet which included cabbage or olives plus black pepper. Both rats are 48 days old. The mean weight of the group which number one represents was 75 g while number two represents a group whose mean weight was 55 g.

As was mentioned before in "Methods and Materials," the studies were done in two identical experiments, one preceding the other by one week. At the end of the study data were pooled and subjected to factorial analysis of variance by the BMD 02V method (Dixon, 1967). The treatments studied were (1) time (first or second week), (2) food (olive or cabbage), (3) condition of the olives and cabbage (fresh or heated), and (4) black pepper (absent or present). The effects of these treatments and their interaction on the weight of the animals and

the number and area of gastric lesions were evaluated. The results of this procedure are presented in Table VI.

Weight. The relationship between experiments 1 and 2 with regards to the weight of the animals under the variable of time was not significant. There were highly significant differences between rats fed spice and no spice ($p < .0005$), between those fed olives and cabbage ($p < .0005$), and between those on fresh versus those on heated olives or cabbage ($p < .005$).

Number of lesions in stomach. The same type of analysis that was done for the weight of the animals was also carried out for the number of lesions. There was no significant difference between rats in the two experiments. There was no significant difference between olive and cabbage fed animals, nor between those receiving heated or fresh factors. The presence of spice in the diet, however, had a marked effect ($p < .0005$) on the number of lesions in the stomach.

Area of lesions. A comparison of the extent of the lesions of the stomach between those animals receiving olives versus those fed cabbage and those that ate fresh versus the ones that had heated cabbage or olives showed no significant difference. On the contrary those animals that were in experiment one had a greater area of lesions than those in experiment two and those receiving spice also had a greater area of lesions ($p < .0005$) than those whose diets were without spice.

Because of this marked difference in lesion area between the animals in experiment one which was done under identical circumstances a week earlier and experiment two, it seems either that there is an

unknown variable operating differently in the two experiments or that this difference is due to variability within groups.

Examples of rat stomachs without and with lesions are shown in Figures 2 and 3.

## GUINEA PIG STUDY

All the guinea pigs showed evidence of abdominal discomfort beginning about ten minutes after they received the injection and persisting for about three hours. During this period the animals were very restless and would lie flat on their bellies, hind-limbs extended and abducted. Occasionally they would roll over on one side or the other. These physical demonstrations of discomfort were very similar to those observed by Eagleton and Watt (1965).

The data collected from the guinea pig study was also subjected to factorial analysis of variance by the BMD 02V method. The treatments studied were (1) food (olive or cabbage) and (2) condition of olives or cabbage (fresh or heated). The effects of these treatments and their interaction on the weight of the animals and the number and area of gastric lesions were also evaluated. Table VII shows the mean values for the analyses while their levels of significance are listed in Table VIII.

Weight. The guinea pigs that were fed the cabbage diet were heavier than those that received olives, but not significantly so.

Number of lesions in stomach. Those receiving cabbage had a significantly larger number of lesions ($p < .05$) than those fed the olive diet.

Area of lesions. A comparison of the extent of the lesions of the stomach between those animals receiving cabbage versus those fed olives and those that ate fresh with those on heated cabbage or olives showed that the guinea pigs receiving cabbage had significantly larger lesions than those that received olives ($p < .025$). The difference between the heated and fresh food component was not significant.

TABLE VI

MEAN VALUES FROM FACTORIAL ANALYSES IN RAT STUDY

| Variables | Categories | Weight | | Number of Lesions | | Area of Lesions | |
|---|---|---|---|---|---|---|---|
| | | Mean | Prob. | Mean | Prob. | Mean ($mm^2$) | Prob. |
| 1. Time | 1. Block | 104.50 | NS | 3.56 | NS | 6.07 | .005 |
| | 2. Block | 101.49 | | 2.34 | | 4.13 | |
| 2. Food | 1. O | 98.06 | .0005 | 2.95 | NS | 4.82 | NS |
| | 2. C | 107.93 | | 2.95 | | 5.38 | |
| 3. Heated or Fresh | 1. F | 100.55 | .005 | 3.36 | NS | 5.11 | NS |
| | 2. H | 105.45 | | 2.55 | | 5.09 | |
| 4. Spice | 1. - | 123.33 | .0005 | 1.75 | .0005 | 3.43 | .0005 |
| | 2. + | 82.67 | | 4.16 | | 6.77 | |

TABLE VII

MEAN VALUES FROM FACTORIAL ANALYSES
IN GUINEA PIG STUDY

| Treatment | No. Animals in Group | Mean Weight of Groups | Mean No. of Lesions | Mean Area of Lesions in Each Group ($mm^2$) |
|---|---|---|---|---|
| Fresh Olives | 9 | 457.67 | 1.44 | 12.05 |
| Heated Olives | 9 | 483.67 | 1.67 | 10.77 |
| Fresh Cabbage | 6 | 473.33 | 4.83 | 102.84 |
| Heated Cabbage | 6 | 503.00 | 3.50 | 45.50 |

TABLE VIII

LEVELS OF SIGNIFICANCE BETWEEN INTERACTIONS
IN GUINEA PIG STUDY

| | Weight | No. of Lesions (Sq. Rt.) | Lesion Area (Sq. Rt.) |
|---|---|---|---|
| Olive vs Cabbage | Not Significant | $p < .05$ | $p < .025$ |
| Heated vs Fresh | Not Significant | Not Significant | Not Significant |

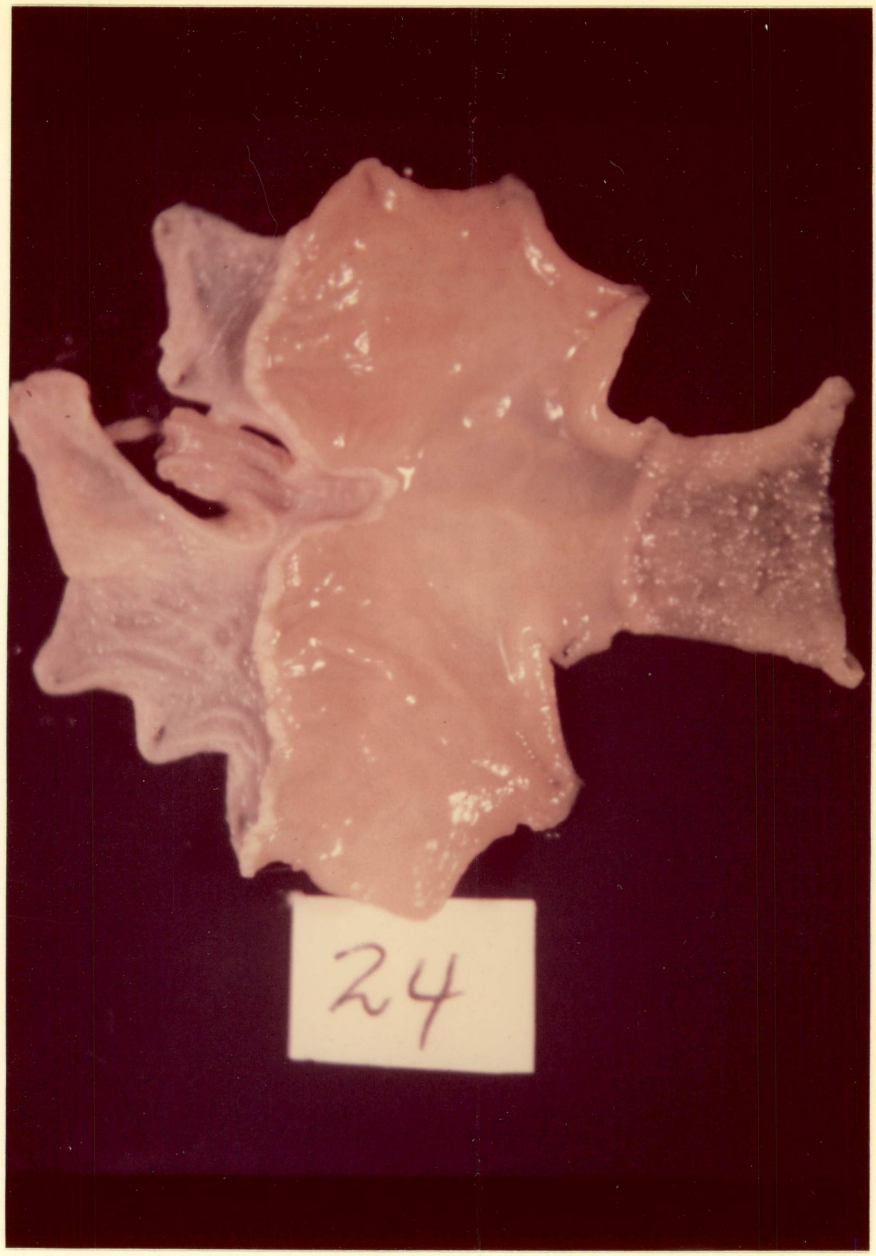


Figure 2. Typical Rat Stomach Without Lesions.


Figure 3. Typical Rat Stomach With Lesions.

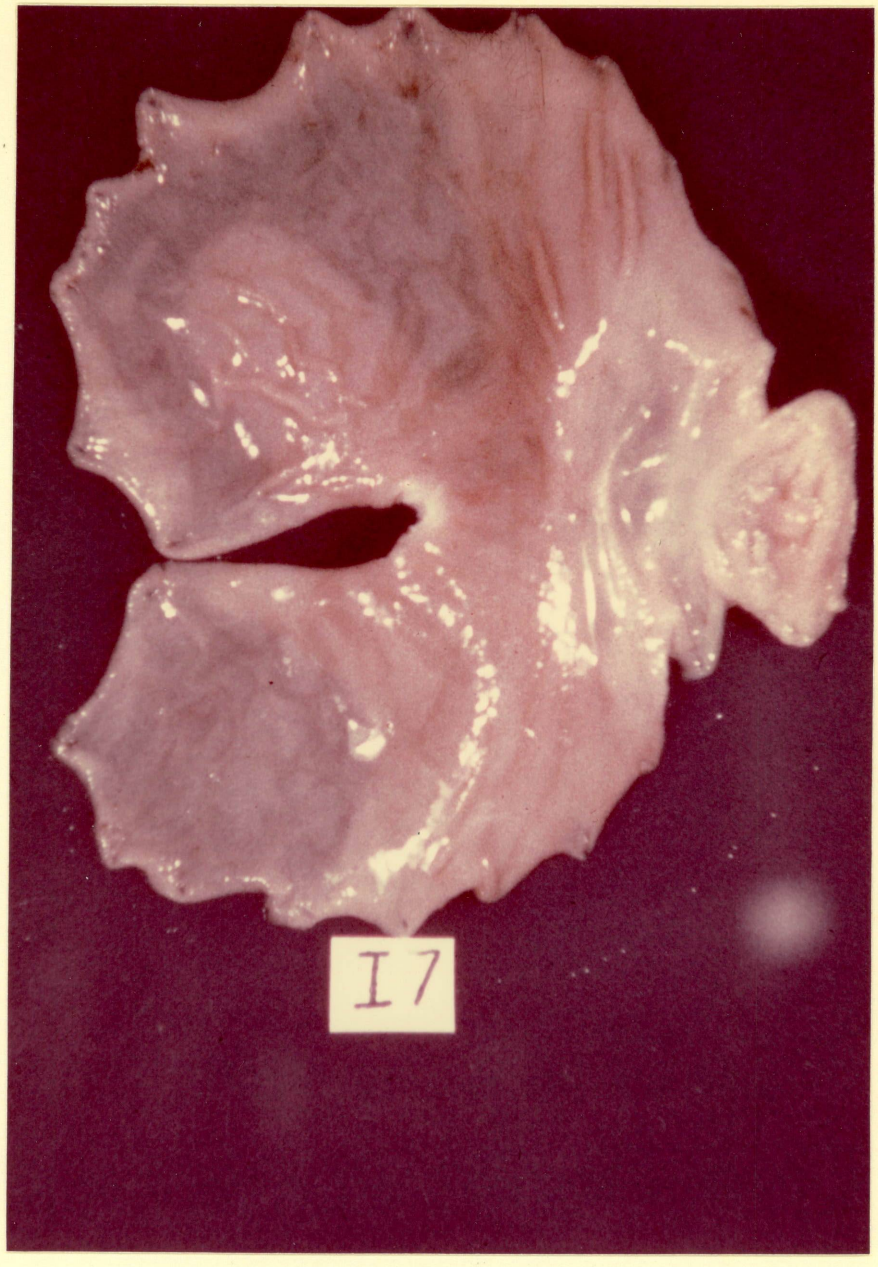


Figure 4. Typical Guinea Pig Stomach Without Lesions.

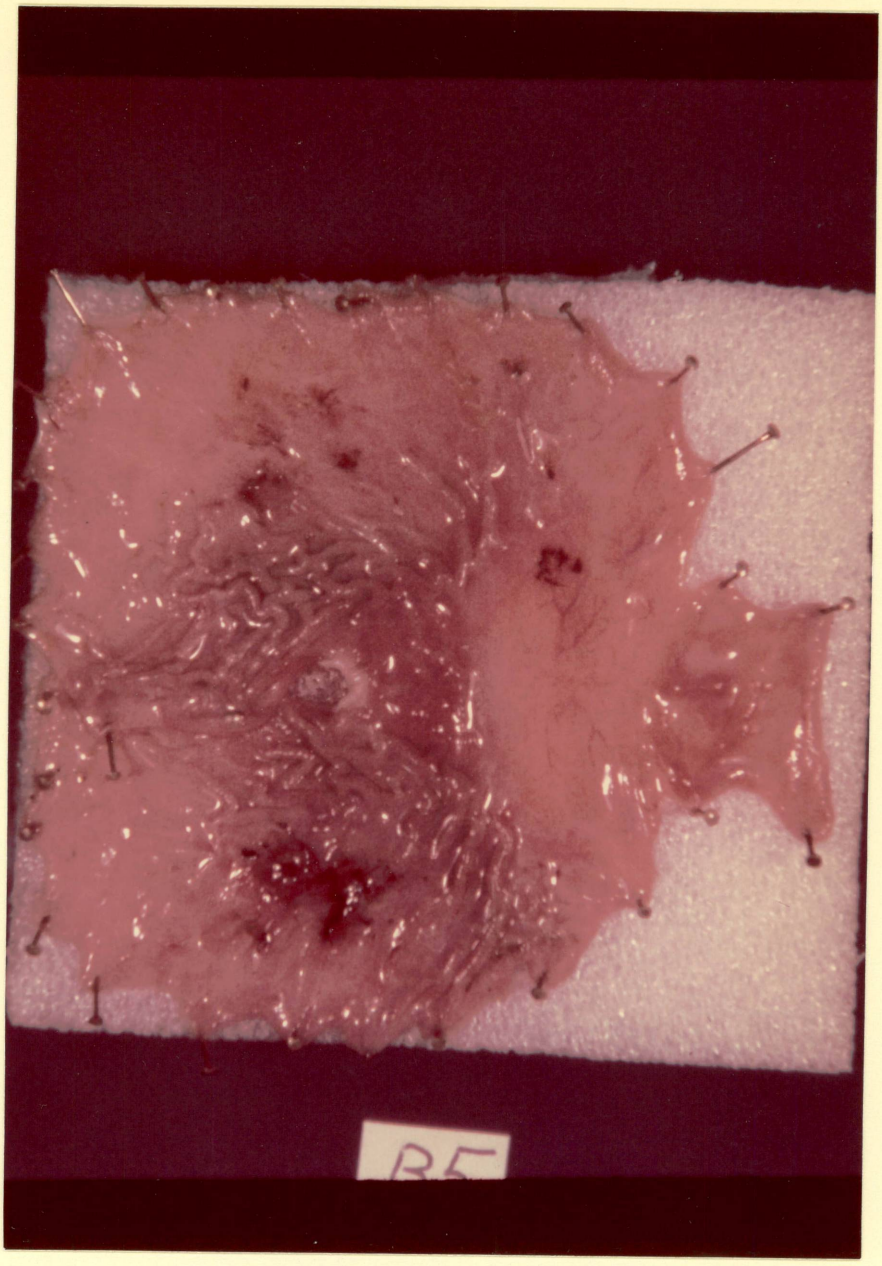

Figure 5. Typical Guinea Pig Stomach With Lesions.

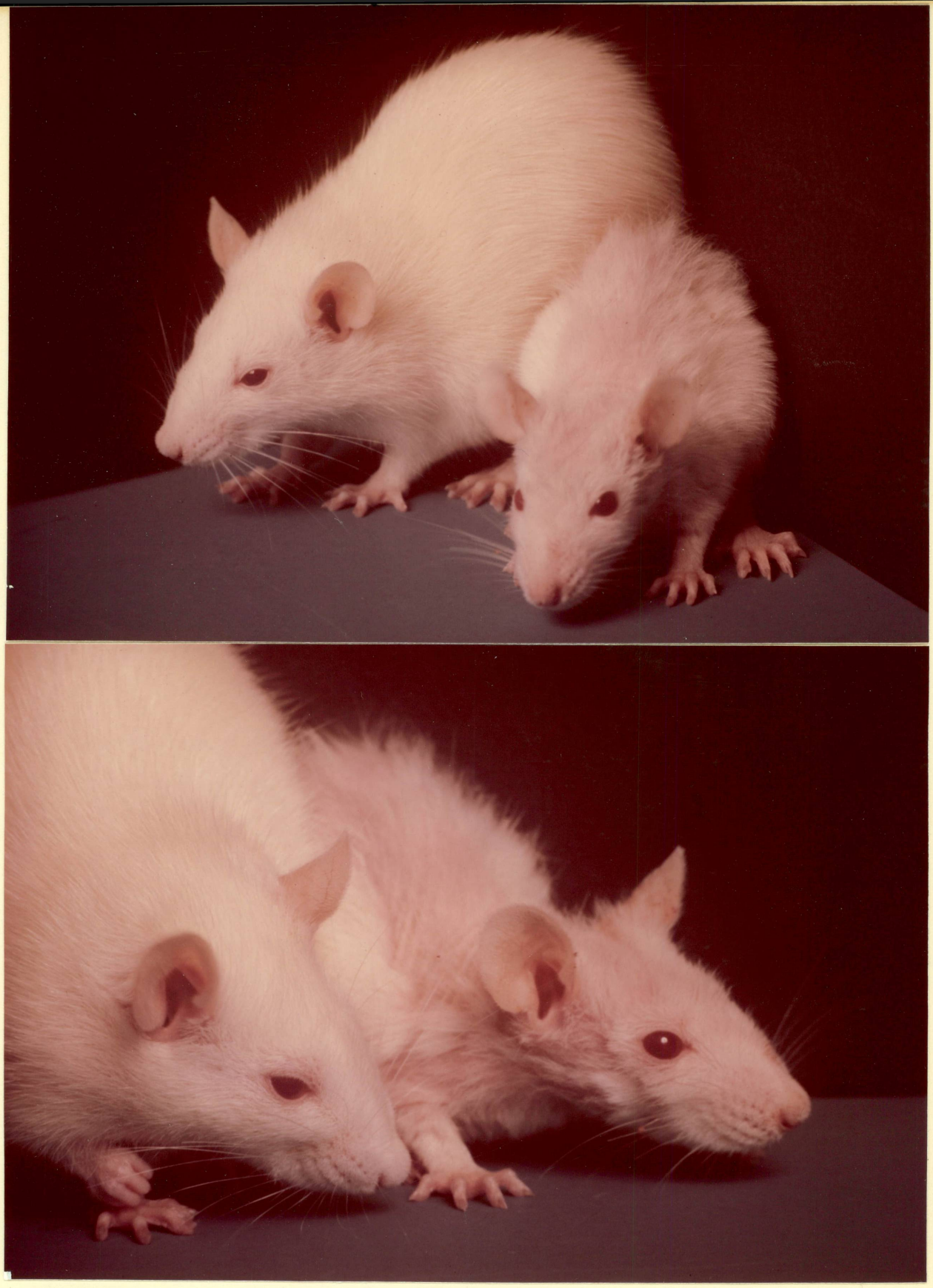

Figure 6. Typical Appearance of Rats Fed Diets With and Without Spice.

## DISCUSSION

These studies support the findings of previous investigators (Sanchez-Palomera, 1951; Schneider, et al., 1956) regarding the ability of black pepper to augment stress-induced peptic "ulceration." The experiments demonstrate that rats receiving black pepper diets had significantly more lesions than those that had no spice. Furthermore, the lesion area in the stomach was significantly greater in the spiced diet groups. We are not aware of other studies which shown the damaging effect of black pepper in the rat.

It was most interesting to find that rats fed the diet supplemented with black pepper had a significantly reduced weight gain as compared to the control groups. In addition, the animals lost much of their hair and were hyperactive when handled for weighing. Since the animals were pair-fed and thus received equal amounts of all nutrients the reason for the reduced weight gain and loss of hair is not immediately apparent. Possible reasons for this phenomenon may include a spice effect either on a reduced intestinal absorptive capacity and/or a decreased utilization of one or more nutrients. Therefore, studies should be conducted to determine the effect of spices on the absorption and utilization of nutrients.

Olive oil (Nesterova, 1963) and cabbage juice (Cheney, 1949; Vargha and Damrau, 1963) have been reported to have ameliorating effects on peptic ulcers in human beings. The active factor seems to be heat sensitive, and present only in unheated samples (Cheney, 1949). In the present studies where both unheated and heated samples were used, there was no beneficial effect of the fresh cabbage or olives

when compared to the heated supplements in either rats or guinea pigs. The studies were not designed to answer the question whether olives or cabbage afford protection against spice feeding as compared with the control diet without either spice or potential protective factor supplementation. Since the olives used in our studies were picked green, the possibility that a protective factor might be developed or present in greater quantity in fully mature fruit should be considered. However, the data do show that olives are significantly more protective in both number and extent of lesions than cabbage with guinea pigs. No such relationship was found with rats.

Since in the guinea pigs heated or unheated olives were significantly more protective against ulcer production than comparable samples of cabbage the data suggest the possibility of factors other than "Vitamin U" as the antiulcer factor. However, it should be pointed out that while both food supplements were fed at the same level on a weight basis, the olives contained twice the solids as cabbage. Further studies with animal and human subjects are needed to determine the role of cabbage and olives in lesion production or prevention.

There are some puzzling results in these studies. There was a tendency of increased numbers and area of lesions in guinea pigs and a significant reduction in weight gain of rats when these were fed the fresh food supplements. The reasons for these results are not known. There was also a significantly greater lesion area in the rats from all the diets in the first experiment as compared to all the animals from the second experiment. The high variability within groups of animals may account for these differences between the two rat experiments.

In some studies (Eagleton and Watt, 1965; Brodie and Hanson,

1960; Brodie, et al., 1963) the method of assessing the degree of "ulceration" was a grading system of - to +++. In this study the direct measurement of each lesion under the microscope gave a more objective quantification of affected areas thus reducing many of the ambiguities of lesion evaluation present in the literature. Most previous workers have reported total numbers or per cents of animals affected and/or total number of lesions. We have clearly shown that the area of involvement is a more realistic parameter than either number of animals or number of lesions. Obviously, three large lesions could involve almost the entire stomach and represent coalition of many smaller lesions; they may also represent much more damage than three punctate lesions.

There has been some controversy among investigators about whether the experimental lesions produced in animals are ulcers (Brodie, 1962). A clear definition of lesions using microscopic and histologic verifications is needed. For the present studies the term lesions has been used throughout. Histological examinations of the stomachs will be done later to determine the depth of the lesions and thus categorize them into erosions or ulcers.

## SUMMARY

Since the social magnitude of peptic ulcer as a national problem is becoming more and more a threat to all people everywhere it is important to study its causes, prevention and cure. To add to the growing body of knowledge concerning the prevention and cure of peptic ulcer, albino rats and guinea pigs were fed fresh and heated supplements of cabbage and water cured olives in addition to nutritionally adequate diets. In addition some of the rats, but not the guinea pigs, received black pepper. At the end of the feeding period the rats were fasted twenty-four hours and then isolated in quantitative stress cages for another twenty-four hours while the guinea pigs were fasted sixteen hours, then injected intraperitoneally with histamine acid phosphate to produce gastric lesions.

Statistical analyses were employed to evaluate the effect of the cabbage, olives, and black pepper on the weight gain, number of lesions and lesion area sustained by the animals. The rats that were fed black pepper gained significantly less weight than those that received no spice. The spice-fed groups also showed scant hair growth down the full length of their bodies, and they had significantly more gastric lesions and larger lesion areas than the nonspice-fed groups. There was no significant difference between those that received cabbage or olives nor did the heating of the supplements cause any substantial change. With the guinea pigs, however, data showed that olives were significantly more protective than cabbage in both number and extent of lesions.

Although the rat study was done in two separate experiments

under identical circumstances the animals in the first experiment had a significantly greater lesion area as compared to all the animals in the second experiment.

More studies with cabbage and olives need to be done before their effect on peptic ulcer can be established.

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# EFFECTS OF DIET AND STRESS ON GASTRIC LESIONS IN ALBINO RATS AND GUINEA PIGS

by

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An Abstract of a Thesis

in Partial Fulfillment of the Requirements

for the Degree Master of Science

in the Field of Nutrition

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ABSTRACT

Studies were conducted to (1) investigate whether any protective factor is present in fresh water-cured olives or fresh cabbage as compared with the heated supplements against stress-induced gastric lesions in rats maintained on a nutritionally adequate diet for six weeks, (2) determine the relative protective factor in water-cured olives as compared with cabbage, (3) study the effect of black pepper in combination with fresh cabbage and olives and heated cabbage and olives in rats that were fed the same nutritionally adequate diet, and (4) investigate the effects of the same dietary components--cabbage and olives--on histamine-induced gastric lesions in guinea pigs fed guinea pig chow for five weeks.

At the end of the feeding period the rats were fasted and then stressed in quantitative stress cages and the guinea pigs were fasted and then injected intraperitoneally with histamine acid phosphate to produce gastric lesions. The effect of the diets on weight gain, number of lesions, and lesion area were analyzed statistically. The rats that were fed black pepper with heated or fresh cabbage or olives had considerably more lesions and a larger lesion area than those that had no spice. They also had a significantly reduced weight gain as compared to the control group. The animals on the spiced diets also lost much of their hair while the control groups maintained white fluffy coats. When the fresh cabbage or olive diets were compared to those with heated supplements no significant effect was found in either rats or guinea pigs. However, in the guinea pig experiments, the data showed that olives were significantly protective in both number and extent of

lesions when compared with cabbage.

The rat study was done in two separate experiments under identical circumstances. However, the animals in the first experiment had a significantly greater lesion area as compared to all the animals from the second experiment. The reason for these results are not known. Further studies with animals and human beings are needed to establish the role of cabbage and olives in lesion prevention.

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