[CANCER RESEARCH 35, 1369-1371, May 1975]

Brief Communication

Importance of the Fecal Stream on the Induction of Colon Tumors by Azoxymethane in Rats[1]

Robert L. Campbell, Dharm V. Singh, and Norman D. Nigro

Wayne State University, School of Medicine, Department of Surgery, Detroit, Michigan 48201

SUMMARY

The effect of the fecal stream on intestinal carcinogenesis with azoxymethane was studied in male rats. Colostomies were performed approximately 2 cm distal to the cecum in 50 Sprague-Dawley rats to produce a 20-cm segment of nonfunctional large bowel; an additional 50 animals were left intact. Each of these groups was divided equally and was fed a normal diet or a diet containing 2% cholestyramine by weight. All animals were given azoxymethane s.c. At the end of 7 months all rats were sacrificed. The animals with colostomies developed significantly fewer tumors in the defunctionalized bowel than did intact animals in the same bowel segment. Cholestyramine appeared to increase the tumor yield in the large bowel of the intact animals but had no effect on the number of tumors in the defunctionalized bowel. Further, the intact animals on both dietary regimens developed a greater number of large tumors in the distal 20 cm of bowel. The results show that the fecal stream alters the carcinogenic activity of azoxymethane in the large bowel of the rat. It also appears that the carcinogen can reach its target tissue by a route other than the fecal stream.

INTRODUCTION

Large bowel cancer is a major problem in this country and is 2nd only to lung cancer as a killer. It has been reported that populations in northwest Europe and North America experience a higher incidence of this cancer than do populations of East Africa, Asia, and South America (5). The diet of persons in these high-risk populations has been found to contain large amounts of animal protein and fat (1). Moreover, migrant studies have shown that groups moving from an area of low risk to one of high risk for colon cancer generally assume the risk of the new area. Thus, there is little doubt from the epidemiological evidence that an environmental factor, probably diet, plays a role in the etiology of colon cancer (1, 7).

From a biochemical standpoint, Hill *et al.* (9) have shown that the feces of persons in areas with a high incidence of colon cancer have a high bile acid content and a highly anaerobic bacterial flora in the colon capable of metabolizing these steroids. It is suggested that, under these conditions, endogenous intestinal carcinogens may be formed from bile acid compounds. It is clear, then, that diet affects the composition of the intestinal contents and therefore the environment with which the bowel mucosa is in contact.

We have previously reported that an increase in the bile acid content of the large bowel by mechanical means (3) or by the use of cholestyramine (13) enhances the carcinogenic activity of azoxymethane in the rat, particularly in the large bowel. We have also found that the addition of beef fat to the diet of the rat has a similar carcinogenic-enhancing effect (15). The purpose of this study is to determine the importance of the presence and composition of the fecal stream in intestinal carcinogenesis with azoxymethane in the rat.

MATERIALS AND METHODS

One-hundred male Sprague-Dawley rats, purchased from Sparton Research Animals, Inc., Haslett, Mich., weighing about 200 g, were used in this experiment.

A colostomy was performed on 50 animals at a point approximately 2 cm distal to the cecum. This procedure produces a segment of defunctionalized colon about 20 cm in length, *i.e.*, with no fecal stream. No operation was performed on the remaining 50 animals. These 100 animals were divided into 4 equal groups. The individual groups were placed on either a normal Purina rat chow diet or a granular Purina rat chow diet containing 2% cholestyramine (Merck, Sharpe, and Dohme, West Point, Pa.) by weight according to the experimental design shown in Chart 1. All animals were housed in individual cages and were fed their respective diets with water *ad libitum*.

Azoxymethane, purchased from Ash Stevens Company, Inc., Detroit, Mich., was prepared in aqueous solution. The rats in all groups were given weekly s.c. injections of the carcinogen at a dosage of 8 mg/kg body weight. The injections were continued until the rats died or were sacrificed. Necropsies were performed on all animals. The distal 20 cm of colon, either functional or nonfunctional, were removed and the number, size, and location of the tumors were recorded. Histological sections were made of representative tumors. The average tumor incidence per animal and the S.E. was determined for each group. The data were analyzed by Student's *t* test (2).

[1] Supported by the Matilda R. Wilson Fund.
Received November 12, 1974; accepted March 3, 1975.

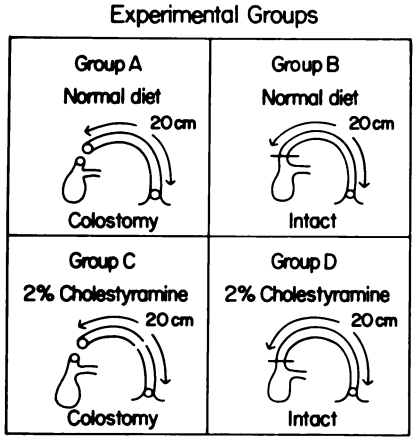

Chart 1. Design of the experimental animal groups.

RESULTS

Several rats in each group died early from pneumonia, leaving 15 animals in Group A and 22 animals in each of Groups B, C, and D. These remaining rats in each group were sacrificed at the end of 7 months. Tumors were found in the distal 20 cm of the large bowel in all experimental groups. Of the animals fed a normal diet, the 15 in Group A developed a total of 34 large bowel tumors in the defunctionalized colon, while the 22 intact animals (Group B) had a total of 108 tumors in the same segment of bowel. The average number of large bowel tumors per rat was 2.3 ± 0.6 and 4.9 ± 0.6, respectively. When cholestyramine was included in their diet, the 22 animals in Group C developed a total of 58 large bowel tumors; in the 22 intact animals in Group D, a total of 136 tumors were found. In these groups, the average number of large bowel tumors per rat was 2.6 ± 0.6 and 6.2 ± 0.7, respectively. These findings are presented in Chart 2.

The size of the tumors found in the various experimental groups varied from 0.1 to 3.0 cm in diameter. As can be seen in Chart 3, the animals with functional colon segments produced a greater number of large tumors, 0.50 to 1.49 cm in size, than did animals with defunctionalized bowel.

Abdominal carcinomatosis was present in 48% of the rats with functional colon but in only 29% of the rats with a defunctionalized colon. Nine tumors, each 0.5 cm in greatest diameter and all located in the distal 20 cm of the colon, were taken from each experimental group and were examined histologically. All the tumors examined were definite adenocarcinomas. The degree of anaplasia varied from well to poorly differentiated. However, there was little or no correlation between tumors from functional or defunctionalized colon and their degree of differentiation.

DISCUSSION

Navarrete and Spjut (12) previously reported that they were unable to induce tumors distal to colostomies in Wistar rats when 3,2'-dimethyl-4-aminobiphenyl was given s.c. These investigators concluded that the diversion of the fecal stream prevented the development of colonic neoplasms by this carcinogen. On the other hand, Wittig *et al.* (20) reported the formation of tumors distal to colostomies when 1,2-dimethylhydrazine was injected into rats.

In our study with Sprague-Dawley rats, we have been able to induce tumors routinely in nonfunctional colon tissues by the s.c. injection of azoxymethane. Thus, it appears that carcinogens affecting the large bowel may have differing routes of action. Azoxymethane and its metabolic precursor, 1,2-dimethylhydrazine (6, 17), appear to be capable of reaching the colonic mucosa by the vascular system, while 3,2'-dimethyl-4-aminobiphenyl (19) apparently acts through the fecal stream. Such findings suggest that the study of blood-carried carcinogens in the investigation of human intestinal cancer may be important.

Our findings indicate that the fecal stream alters the number of tumors produced by azoxymethane in the colon. The average number of tumors per animal was significantly increased in the functional colon over that observed in the animals with colostomies. Further, larger tumors were found in the colons of intact animals than in those with the fecal stream diverted. The tumor incidence in the nonfunctional colon was not affected by the addition of 2% cholestyramine to the diet. However, in the functional colon, cholestyramine produced a small increase in tumor incidence but not to a significant degree nor to the level previously reported (13). These findings suggest that the presence and possibly the composition of the fecal stream are important factors in the carcinogenic activity of azoxymethane. The results lend experimental support to the epidemiological evidence relat-

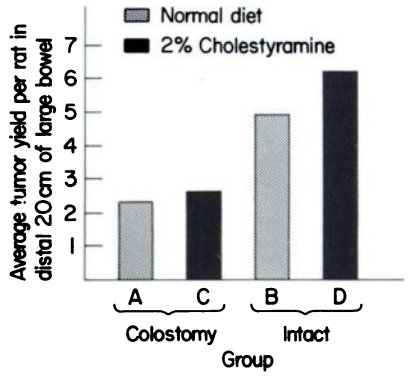

Chart 2. Average tumor yield/rat in the distal 20 cm of large bowel for each experimental group. The differences observed in the tumor yield between Groups *A* and *B* as well as between Groups *C* and *D* are significant ($p < 0.005$ and < 0.0005, respectively).

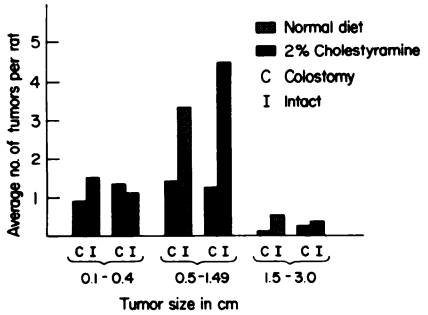

Chart 3. Average number of tumors per rat in the distal 20 cm of large bowel as a function of tumor size (greatest diameter). Significant differences in the number of tumors 0.5 to 1.49 cm in diameter are observed between colostomy and intact animals on each dietary regimen (normal diet, colostomy *versus* intact, $p < 0.005$; 2% cholestyramine diet, colostomy *versus* intact, $p < 0.0005$).

ing diet and fecal composition to the high incidence of colon cancer in certain population groups.

The mechanism of the altering effect of the fecal stream upon carcinogenesis in our animal model is not entirely clear in the present study. Although we have found azoxymethane or a metabolite to be capable of reaching the colonic mucosa by the vascular system, it is possible that this carcinogen also acts through the fecal stream. Thus, the decrease in tumor formation in the rats with the colostomy may be the result of less carcinogen reaching the colon. On the other hand, the colonic mucosa of nonfunctional bowel may be less susceptible to the carcinogen, thus causing a lower tumor incidence.

However, other alternatives should be considered. It has been suggested that bile acids are involved in the formation of intestinal cancer (8, 9). We have found previously that the formation of intestinal tumors in the rat by azoxymethane can be enhanced by the feeding of cholestyramine (13) or by the transplantation of the bile duct to the mid-small intestine (3). In these studies, an increased amount of total bile acids was found in the feces and colonic tissues of the experimental animals (14). In this context, the lower tumor incidence found in nonfunctional colon may be the result of a diminished level of luminal bile acids that may be acting as promoting agents in the carcinogenic process.

The bacteria of the gut have been reported to be important to the carcinogenic activity of several intestinal carcinogens (4, 10, 11). The diversion of the fecal stream by a colostomy should reduce or at least alter the microflora present in nonfunctional colon. Reddy *et al.* (18) have recently reported that microflora may play a modifying role in 1,2-dimethylhydrazine carcinogenesis. Thus, the difference in tumor incidence observed between functional and nonfunctional colon may be the result of the alteration in microflora that may be important in the carcinogenic process of azoxymethane.

The importance in cellular proliferation in carcinogenesis has recently been reviewed by Oehlert (16). In the study of chemically induced skin cancers, a high cellular proliferative activity during the initial application has been found to enhance the effect of many carcinogens. If the presence or absence of the fecal stream influences the proliferative activity of intestinal epithelial cells, it may be that the effect of azoxymethane has been influenced in a manner similar to skin carcinogens.

The modifying effect of the fecal stream upon the carcinogenic activity of azoxymethane may be due to several factors, many of which may be interrelated. Thus, the study of intestinal carcinogenesis may be a more complex problem than originally thought. We are continuing to investigate some of these factors, their interrelationships, and their influence on intestinal carcinogenesis.

ACKNOWLEDGMENTS

We would like to acknowledge the excellent technical assistance of Dr. C. Chomchai, Kenneth Kohn, Dale Miller, and Diana Yu. We also wish to thank Dr. M. S. Pak for the histological work and Emily Cooper for excellent secretarial assistance.

REFERENCES

1. Berg, J. W., Howel, M. A., and Silverman, S. J. Dietary Hypothesis and Diet-related Research in the Etiology of Colon Cancer. Health Service Rept., *88:* 915-924, 1973.
2. Beyer, W. Handbook of Tables for Probability and Statistics, Ed. 2. Cleveland, Ohio: Chemical Rubber Co., 1968.
3. Chomchai, C., Bhadrachari, N., and Nigro, N. D. The Effect of Bile on the Induction of Experimental Intestinal Tumors in Rats. Diseases Colon Rectum, *17:* 310-312, 1974.
4. Cole, J. W. 2',3-Dimethyl-4-aminobiphenyl: Absence of Carcinogenicity in Germ-free Rats. Quoted by Wynder, E. L., Kajitani, T., Ishikawa, S., Dodo, H., and Takano, A. Environmental Factors of Cancer of the Colon and Rectum II. Japanese Epidemiological Data. Cancer, *23:* 1210-1220, 1969.
5. Doll, R. The Geographical Distribution of Cancer. Brit. J. Cancer, *23:* 1-8, 1969.
6. Druckrey, H. Organospecific Carcinogenesis in the Digestive Tract. *In:* W. Nakahara, S. Takayama, T. Sugimura, and S. Odashima (eds.), Topics in Chemical Carcinogenesis, pp. 73-103. Tokyo: University of Tokyo Press, 1972.
7. Haenszel, W., Berg, J., Segi, M., Kurihara, M., and Locke, F. Large Bowel Cancer in Hawaiian Japanese. J. Natl. Cancer Inst., *51:* 1765-1779, 1973.
8. Hill, M. J., and Aries, V. C. Faecal Steroid Composition and Its Relationship to Cancer of the Large Bowel. J. Pathol., *104:* 129-139, 1970.
9. Hill, M. J., Drasar, B. S., Aries, V., Crowther, J. S., Hawksworth, G., and Williams, R. E. O. Bacteria and Etiology of Cancer of the Large Bowel. Lancet, *1:* 95-100, 1971.
10. Laqueur, G. L. Contribution of Intestinal Macroflora and Microflora to Carcinogenesis. *In:* W. J. Burdette (ed.), Carcinoma of the Colon and Antecedent Epithelium, pp. 305-313. Baltimore: Williams & Wilkins, Inc., 1970.
11. Laqueur, G. L., and Spatz, M. Toxicology of Cycasin. Cancer Res., *28:* 2262-2267, 1968.
12. Navarrete. A., and Spjut, H. J. Effect of Colostomy on Experimentally Produced Neoplasms of the Colon of the Rat. Cancer, *20:* 1466-1472, 1967.
13. Nigro, N. D., Bhadrachari, N., and Chomchai, C. A Rat Model for Studying Colonic Cancer: Effect of Cholestyramine on Induced Tumors. Diseases Colon Rectum, *16:* 438-443, 1973.
14. Nigro, N. D., Sardesai, V. M., Chomchai, C., Campbell, R. L., and Provido, H. Bile Acid Content of Intestinal Tissue and Feces during Azoxymethane Carcinogenesis in the Rat. Federation Proc., *33:* 260, 1974.
15. Nigro, N. D., Singh, D. V., Campbell, R. L., and Pak, M. S. Effect of Dietary Beef Fat on Intestinal Tumor Formation by Azoxymethane in Rats. J. Natl. Cancer Inst., *54:* 439-442, 1975.
16. Oehlert, W. Cellular Proliferation in Carcinogenesis. Cell Tissue Kinet., *6:* 325-335, 1973.
17. Preussmann, R., Druckrey, H., Ivankovic, S., and Hodenberg, A. V. Chemical Structure and Carcinogenicity of Aliphatic Hydrazo, Azo, and Azoxy Compounds and of Triazenes, Potential *in Vivo* Alkylating Agents. Ann. N. Y. Acad. Sci., *163:* 697-716, 1969.
18. Reddy, B. S., Weisburger, J. H., Narisawa, T., and Wynder, E. L. Colon Carcinogenesis in Germ-free Rats with 1,2-Dimethylhydrazine and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Cancer Res., *34:* 2368-2372, 1974.
19. Spjut, H. J., and Noall, M. W. Experimental Induction of Tumors of the Large Bowel of Rats. A Review of the Experience with 3,2'-Dimethyl-4-aminobiphenyl. Cancer, *28:* 29-37, 1971.
20. Wittig, D., Wildner, G. P., and Ziebarth, D. Der Ein Fluss der Ingesta auf Kanserisierung des Rattendarms durch Dimethylhydrazin. Arch. Geschwulstforsch., *37:* 105-115, 1971.