Carcinoma of the Colon and Antecedent Epithelium
Report of a Symposium

*Walter J. Burdette, M.D.

Under the auspices of the American Cancer Society, a symposium workshop on carcinoma of the colon and antecedent epithelium was held in Houston as part of an assessment of the program of the American Cancer Society carried out by their Council on Analysis and Projection, and devoted to the better understanding of carcinoma of the colon and its management. It was quite clear that a fresh breeze is dispelling relative inactivity in an important field and that a surprising number of approaches not exploited fully are now open to the investigator interested in this field. This type of neoplasm contributes the second largest number of expected deaths in the United States annually at the present time. Apparently, the outlook for saving a greater percentage of patients with ablative techniques is not hopeful and therefore considerations of etiology, diagnosis, chemotherapy and immunotherapy discussed at this meeting represent the best chance for improving results. Fortunately, some of the returns from the laboratory are encouraging, such as those indicating that carcinoembryonic antigens are unique for adults with neoplasms of the alimentary tract and are not shared by those without these neoplasms.

Clinical Cancer of the Colon
The theme for the meeting was set by an introductory paper by W. J. Burdette,
who emphasized that attention must be directed toward the epithelium of the colon antecedent to the neoplastic change, its inborn susceptibilities, and its response to various agents. He reviewed neoplastic diseases of the colon, the difference in behavior of various types of polyps (many are benign) and carefully defined adenocarcinoma of colorectum and its behavior as the target for intensive investigation. The experience at The University of Texas M. D. Anderson Hospital with 2,245 adenocarcinomas of the colon was then presented along with a review of the present state of information about heritable cancers. He pointed out that in at least four heritable syndromes, colonic carcinomas due to single dominant genes may be identified, whereas animal models with such precise definition of genetic susceptibility do not exist, and urged that this favorable material be used in experiments to understand pathogenesis. Multiple polyposis and Gardner's syndrome appear with sufficient frequency because of relatively favorable equilibrium between mutation rate and selection pressure to provide enough candidates for extensive studies. Less frequently, an occasional kindred with dominant pattern for propagation of adenocarcinoma of the colon alone or in conjunction with other stigmata of a pleiotropic phenotype also provides an excellent opportunity for investigating properties of colonic mucosa destined to become malignant.

J. S. Spratt reported gross rates of growth of the neoplasms of the large intestine and rectum determined from roentgen studies with barium. He found that the diagnosis of adenocarcinoma is quite likely in all lesions of colon and rectum larger than 2 cm. When successive measurements are available from roentgen examination, increments in chordal dimensions greater than 0.003 mm./day are compatible with the diagnosis of carcinoma also. These findings are valuable because of the realization in recent years that most adenomatous polyps are not precancerous.

W. M. Keynes reviewed current results in the management of colonic and rectal cancer and concluded that present methods, consisting chiefly of operative techniques, need expansion in other directions if results are to be improved substantially. He mentioned studies on etiology, examination of epidemiology of colonic mobility, and efforts to obtain earlier diagnosis through examinations for unique antigens as important subjects for expanded research and called attention to the controversial nature of preoperative antibiotics, use of chemotherapy as an adjunct to operation, and the failure of routine procedures for screening and studies of exfoliated cells to improve diagnosis very much in the past.
Antigens Unique for Colonic Cancer and Embryonic Epithelium of the Gut

The determination of α-fetoprotein in the serum of patients with primary hepatic cancer and in monkeys with hepatomas following treatment with N-nitrosodiethylamine may be considered a prototype for testing the value of antigens occurring in fetal and neoplastic tissues as a screening device for cancer. P. Carbone reported that 40 percent or less of American patients with primary hepatic cancer had α-fetoprotein in their serum. When compared with results for patients elsewhere, broad differences in α-fetoproteins in patients with hepatic cancer occurred throughout the globe, possibly as a reflection of different etiologies in different regions.

P. Gold reported the results of studying 200 colonic adenocarcinomas and 1,500 specimens from other tissues both normal and diseased. Antigens identical with those found to be specific for colonic cancers were detected in specimens of primary and metastatic cancer derived from the epithelium of the digestive system originating in the entoderm. They were not present in normal tissues or benign lesions of the gastrointestinal tract or in primary cancers in other tissues. The same carcinoembryonic antigens were found in embryonic and fetal gut, pancreas and liver during the first 2 trimesters of gestation. With a radioimmunoassay, all except 1 of 34 patients with adenocarcinoma of the rectum exhibited detectable levels of circulating CEA.*

The highest concentrations of CEA are found in cancers of the large intestine.

Results from testing the sera of 212 individuals for circulating antibodies against CEA by the technique of bisdiazotized benzidine hemagglutination disclosed that samples from 30 of the 43 patients with primary cancer arising in digestive organs showed positive hemagglutination titers against CEA. With the use of both fluorescein-conjugated and ferritin-conjugated anti-CEA antiserum, CEA was localized more peripherally than the trilamellar plasma membrane. Of 46 women tested during pregnancy and immediately postpartum, 28 exhibited circulating anti-CEA antibodies, and 2 patients with diseases other than cancer of the digestive tract were positive.

The discussion of tumor-specific antigens by P. Burtin was characterized by restraint. He pointed out that an antigen apparently specific for a tumor and absent from a corresponding normal organ may be present in other organs and that the specificity of tumor-specific antigens can be affirmed only after examination of a variety of tumors from different organs. Also a study of fetal organs is indispensable in order to gain insight into the origin of these antigens. He described three antigens extracted from colonic neoplasms with perchloric acid, two with β mobility (internal and external), and one with mobility of an α-globulin. The antigen giving a β internal line was identical with CEA originally described by P. Gold and S. O. Freedman. In seven colonic neoplasms, the a H-globulin was tentatively identified as ferritin by D. Buffe. The technique described by Gold for detecting circulating antibodies against CEA was not at first reproducible, but later better results were obtained, although some sera from patients without colonic cancer gave positive results including two patients with gastroduodenal ulcer and one with Crohn's disease.

B. S. Kronman also found a qualitative antigenic difference between normal colon and adenocarcinoma of the colon in nine patients. Kronman reached the usual conclusion about the great likelihood that colonic adenocarcinoma will supervene when ulcerative colitis exists for a long time. He also reviewed carefully the relationship between ulcerative colitis and autoimmune mechanisms but failed to find convincing evidence that ulcerative colitis is caused by autoimmune processes although immunological epiphenomena are present in the disease.

* The abbreviation CEA denotes carcinoembryonic antigens.
Immunotherapy

Immunity against human colonic carcinomas mediated by lymphocytes in peripheral blood was tested by colony inhibition by I. Hellström and K. E. Hellström who found that exposure to either autochthonous or allogeneic lymphocytes from patients with colonic cancer inhibited the formation of colonies of neoplastic cells from colon. A protective effect was noted when sera from certain patients was added to the cultures. These investigators concluded that adenocarcinomas of the colon possess common specific tumor antigens but were unwilling to state the exact relationship to CEA or their possible usefulness in future immunotherapy. In discussing immunotherapy, K. E. Hellström expressed the view that sufficient experimental evidence exists to support the philosophy of immunotherapy for adenocarcinoma of the colon, but clinical trial should not be undertaken unless the favorable circumstance of small numbers of neoplastic cells is present and the risk of enhancement is justified by the condition of the patient and his circumstances.

Cellular Kinetics and Therapy

M. Lipkin reported differences in durations of phases of the cell cycle, the rate of cellular replication, and the regulatory control of cellular differentiation as the epithelium of the colon changes from the normal to the neoplastic state. Repeated biopsies of colonic mucosa revealed proliferating cells to be located in the lower two thirds of the walls of the crypts with migration and extrusion at the surface of the mucosa. Differentiation of these cells was accompanied by changes in level of enzymes that catalyze steps in the intermediary metabolism of nucleic acid, and decrease in comparative amounts of the enzymes was associated with the onset of neoplastic growth.

E. E. Deschner reported results obtained with cultures of colonic epithelium in vitro of brief duration. She found that incorporation of tritiated thymidine in vitro paralleled similar studies on colonic mucosa in vivo, and different patterns of incorporation were found when studies were made on colonic adenomatous polyps, villous papillomas and areas of hyperplasia. Ordinarily epithelial cells below the surface of the mucosa synthesized DNA and greater amounts of RNA and protein than mature cells on the surface of the normal mucosa, but cells located at the surface of the crypts in hyperplasias, adenomatous polyps and villous papillomas incorporated thymidine and more leucine and uridine than cells deeper in the crypts. This altered behavior also applied to epithelium adjacent to neoplasms although it appeared histologically normal.

The behavior of cells lining the crypts both of the colon and jejunum after radiation was described by H. R. Withers who used a technique consisting of observing the behavior of colonies of epithelium on the mucosal surface of intestine regenerating after radiation while exteriorized. Subsequent radiation after replacement of the bowel within the peritoneal cavity led him to conclude that regeneration of the population of stem cells is achieved by decreasing the mean time of the generation cycle and switching off terminal differentiation. Because the cells in the villi persist after depopulation of the cryptal lining and subsequently the stem cells regenerated in the cryptal column at the same time involution of the villus occurs, it is possible that the villus may regulate the differentiative kinetics of the stem cells originating in the walls of the crypts.

Different modes of cellular proliferation and metabolism were described for solid tumors in comparison to ascitic tumors by I. F. Tannock. The latter obtain much of their energy by glycolysis and the durations of phases in the cell cycle are comparatively long. In contrast, the neoplastic cells in solid tumors develop a blood supply but die soon after becoming hypoxic. Although these hypoxic, slowly proliferating cells may have only a limited lifetime in tumors that are not treated, they may survive chemotherapy that will reach
other cells that are better nourished. This cell is resistant to radiotherapy because it is hypoxic and to many drugs because it proliferates slowly and is removed from adequate circulation. Thus it is apparent that the relationship between kinetics and responses to treatment is exceedingly complex in solid neoplasms and therefore merits recognition as an unique problem to be studied when management of neoplasms such as those in the colon is considered.

The use of compound sponges composed of cellulose and collagen for culturing tissues was described in detail by J. Leighton who inoculated both fresh and preserved specimens of neoplasms onto this three-dimensional framework and found that colonic cancer along with a number of other neoplasms will grow alone and combined with various tissues derived from embryos and other sources. This technique liberates the investigator from the limitations of the planar surface characteristic of usual techniques for studies in vitro, and the new sponge described seems to be superior to the sponge used previously. A very ingenious use of everted segments of human fetal gut requiring no blood supply in culture chambers and supporting the growth of tissues up to several mm. in size on the mucosa was described by J. Folkman. The method seems to hold some promise for expanding studies of both intestinal mucosa and adenocarcinomas of the colon. When the methods used by Leighton and Folkman are combined with those described by Withers, Lipkin and Deschner for following cellular kinetics of intestinal epithelium, a formidable array of techniques is displayed for study, although the usual limitation for the length of survival of ordinary samples in culture still prevails.

R. O. Johnson reviewed the implications of cellular kinetics for the treatment of colonic cancer in the light of adjuvant chemotherapy for adenocarcinoma of the colon and rectum with 13 different drugs studied as part of the central clinical program for evaluating drugs of the National Cancer Institute. The numbers of patients used for evaluating each drug varied from 11 to 85, and the responses ranged from 7 to 50 percent in 9 of the groups with no responses to the remaining 4 drugs. The fact that objective responses of brief duration or longer occurred in only 57 out of 407 cases appears discouraging, but a philosophy for chemotherapy has evolved which leans heavily on cellular kinetics. With the possible exception of antimetabolites not exhibiting a linear dose response, Johnson affirmed that there seems to be a good chance that appropriate drugs alone or in combination may alter the course of colonic carcinoma favorably in the future if adapted to behavior and characteristic of the specific neoplasms treated.

Carcinogenesis

H. Spjut and M. Noall reported on continuation of their studies in which neoplasms of large and small intestines have been induced with 3, 2-dimethyl-4-aminobiphenyl in white Wistar rats. The benign and malignant neoplasms induced besides those in the bowel were numerous and diverse, but the numbers and sites of neoplasms could be altered by changing the schedules followed in administering the compound. For example, neoplasms of the bowel were induced by a comparatively small dose and short course in male but not female rats. Also the distal colon contained no tumors when excluded from the fecal stream by colostomy. J. W. Cole continued the discussion by comparing the neoplasms of the colon induced in experimental animals by the aminobiphenyls to those appearing spontaneously in the clinical population. He reported a very close correlation between the appearance macroscopically and microscopically of polyps and adenocarcinomas in patients and laboratory animals. However, neither he nor Spjut has observed actual transition between polyps and adenocarcinomas.

H. Druckrey, continuing the important work he and his group are doing on nitrosamines, reported the induction of multiple adenocarcinomas of the colon.
and rectum in all BD rats treated with either 7 or 21 mg./kg. 1, 2-dimethyl-hydrazine and either 6 or 12 mg./kg. azoxy methane injected s.c. weekly. The time for induction ranged between 184 and 380 days depending on the dosage. The difficulties in obtaining neoplasms of the colon within the recent past makes the colontropy described most welcome news. Apparently, the organs in which neoplasms develop depend on the nature of the alkyl groups. An incidental finding in the course of these studies was the discovery that 1-methyl-2-benzylhydrazine is a very potent neurotropic carcinogen.

The role of microorganisms in carcinogenesis is of particular interest in relation to pathogenesis of cancer of the colon where the largest volume of bacteria in the body is normally found. G. L. Laqueur recounted his experience with cycasin and gave a very effective review of the role of the microflora of the gut in carcinogenesis. Bacterial organisms capable of hydrolyzing β-glucosides hydrolyze cycasin to its aglycone, methylazoxy methanol, which is the carcinogen. This explains why neoplasms of lower colon and rectum that occur frequently when cycasin is administered orally do not appear when the same experiment is repeated with axenic rats. He also pointed out that the metabolism of carcinogenic hydrocarbons following oral ingestion and of N-hydroxy metabolites of carcinogenic amines are probably altered by microflora in the gut.

R. E. Billingham and W. K. Silvers emphasized the importance of epithelial-mesenchymal interactions that control proliferative activity of germinal layers, and suggested that they operate throughout life. Because epithelial specificities of skin and probably of the colon are determined at least in part by diffusible agents of mesenchymal origin, the implication follows that derangements of these control mechanisms should not be neglected in considerations of carcinogenesis in the case of colonic neoplasms.

Finally, P. N. Magee summarized the current status of chemical carcinogens in terms of the ultimate carcinogen and metabolic pathways, emphasizing the application of information from the laboratory now available for colonic neoplasms to the clinical situation. The changing spectrum of drugs, natural products in foodstuffs, and environmental materials to which the population is exposed requires reevaluation of extrinsic agents and their possible role in the pathogenesis of neoplasms of colon and rectum.

Transformation of Fibroblasts from Patients with Colonic Cancer

D. Mukerjee and W. J. Burdette determined the susceptibility of fibroblasts from the colon and from skeletal muscles of patients with colonic adenocarcinoma to transformation after exposure to simian papovavirus 40. In four cases duplicate samples showed a suggestive increase in frequency of transformation when compared to that for fibroblasts from patients without carcinoma of the colon.

Chromosomal Abnormalities in Cancer of Colon and Rectum

The karyotypes of 11 primary neoplasms of the large bowel were outlined in detail by H. A. Lubs who found chromosomal abnormalities in 10 patients with malignant or premalignant lesions. The single benign adenomatous polyp he studied had a normal karyotype. The colonic tumors were complex cytogenetically, and distinct lines of cells seemed to evolve in them. An atypical acrocentric chromosome was present in three of the seven adenocarcinomas. The suggestion is made by Lubs that adenocarcinomas of the colon with large numbers of chromosomes are more likely to metastasize than those near diploidy.

P. Nowell pointed out that there has been no consistent chromosomal change in solid tumors comparable to that of the Ph1 chromosome in chronic granulocytic leukemia. Although marker chromosomes have not been identified in them, most solid tumors have abnormal
chromosomal patterns, and the extent of these changes usually corresponds to the degree of progression of the tumor. Apparently, there is some selective advantage to cells with the chromosomal abnormalities in view of the frequency with which a single stem line characterizes the tumor in all except the very early stages of its development. Relating the chromosomal abnormalities to specific alterations in genic action is beyond the scope of present methodologies. However, the numerous deviations from the normal karyotype do suggest that corresponding biochemical characteristics of neoplastic cells are sufficiently diverse to discourage the idea that there will be a uniform response of all tumors from a specific site such as colon to any given chemotherapeutic agents. On the other hand the presence of specific antigens in tumors encourages the hope that immunotherapy may become useful for an entire group of neoplasms.

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Participants

Dr. Rupert E. Billingham
School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Dr. Walter J. Burdette
University of Texas
M.D. Anderson Hospital and Tumor Institute
Houston, Texas

Dr. Pierre Burtin
Institut de Recherches Scientifiques sur le Cancer
Villejuif, France

Dr. Paul Carbone
National Cancer Institute
Bethesda, Maryland

Dr. W. Chang
McGill University
Montreal, Canada

Dr. J. W. Cole
Yale University School of Medicine
New Haven, Connecticut

Dr. A. R. Curret
University of Wisconsin Medical Center
Madison, Wisconsin

Dr. Eleanor E. Deschner
Cornell Medical Center
New York, New York

Dr. H. Drueckey
Max-Planck-Institut für Immunbiologie
Freiburg, West Germany

Dr. Harry Eagle
Albert Einstein College of Medicine
Bronx, New York

Dr. Judah Folkman
The Children's Hospital Medical Center
Boston, Massachusetts

Dr. Phil Gold
Montreal General Hospital
Montreal, Canada

Dr. E. Couley Hammond
The American Cancer Society, Inc.
New York, New York

Dr. Karl Hellström
The School of Medicine
University of Washington
Seattle, Washington

Dr. Ingegard Hellström
The School of Medicine
University of Washington
Seattle, Washington

Dr. Anthony R. Imondi
Cornell Medical Center
New York, New York

Dr. Robert O. Johnson
University of Wisconsin Medical Center
Madison, Wisconsin

Dr. W. Milo Keynes
The University of Oxford
Oxford, England

Dr. Barry S. Kronman
National Cancer Institute
Bethesda, Maryland

Dr. Gert L. Lauquier
National Cancer Institute
Bethesda, Maryland

Dr. Joseph Leighton
University of Pittsburgh
Pittsburgh, Pennsylvania

Dr. Martin Lipkin
Cornell Medical Center
New York, New York

Dr. Herbert A. Lubs
Yale University School of Medicine
New Haven, Connecticut

Dr. Colin MacLeod
The Commonwealth Fund
New York, New York

Dr. Peter Magee
Middlesex Hospital Medical School
London, England

Dr. Debas Mukerjee
University of Texas
M.D. Anderson Hospital and Tumor Institute
Houston, Texas

Dr. Matthew Noall
Baylor University School of Medicine
Houston, Texas

Dr. Peter Nowell
University of Pennsylvania
Philadelphia, Pennsylvania

Dr. Van R. Potter
University of Wisconsin
Madison, Wisconsin

Dr. Willys K. Silvers
School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Dr. Harlan Spjut
Baylor University School of Medicine
Houston, Texas

Dr. John S. Spratt, Jr.
Ellis Fischel State Cancer Hospital
Columbia, Missouri

Dr. Ian Tannock
Institute of Cancer Research
Surrey, England

Dr. John Weisburger
National Cancer Institute
Bethesda, Maryland

Dr. H. Rodney Withers
University of Texas
M.D. Anderson Hospital and Tumor Institute
Houston, Texas