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CANCER OF THE LARGE INTESTINE IN MAN: PROGRESS AND PROBLEMS

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Large bowel cancer is one of the more common forms of malignant diseases in Europe and North America. For example, in 1971, it had been estimated there would have been 75,000 new cases and 46,000 deaths from this tumour in the United States. The pattern of incidence in Western Europe follows that observed in North America, whilst it is remarkably low in the countries of the Third World. The death rate of 19/100,000 males has remained fairly static for a number of years, and ranks second to lung cancer as a cause of death directly attributable to cancer. It would now seem that the survival rate following surgery has reached a *plateau*, the prognosis being directly related to the extent of spread of the tumour at the time of presentation [5, 37].

DUKES classification [14] conveniently describes the extent of the tumour at presentation and will be used as the basis of discussion in this paper. Dukes divided tumours of the colon and rectum into 3 groups that he called A, B and C, which were defined as follows:

A. confined to the mucosa and sub-mucosa;

B. invasion through the bowel wall without involvement of the regional nodes;

C. metastases to the regional nodes.

Since this classification was introduced, it has been customary to add a 4th group D to describe patients with distant metastases.

The 5-year survival for these various groups being as follows: A = 61-81 %; B = 25-64 %; C = 6-28 %[37]. Patients with distant metastases at first presentation very rarely survive more than 5 years. Hence it can be seen that curability is closely linked to the presence or absence of metastases to lymph nodes. Approximately 25 % of all patients presenting have distant spread and fall into the D group. The A cases are probably less than 10 % of the total load, the remainder being B's and C's, being

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divided in about equal numbers. Apart from the immediate involvement of the peritoneum, the distant sites of metastases are predominantly in the liver and the lung, with the less common sites being in the bone, brain, adrenal glands and kidney. The duration of life from the time of discovery of either an unresectable lesion or a distant metastasis can vary enormously. On average it is about 10 months, with a range of 4 weeks to over 6 years [37]. This factor clearly indicates that the behaviour of the disease follows a widely different pattern from one individual to the next. It will be seen that this variability is reflected in many of the laboratory and biological investigations made on patients bearing colo-rectal tumours and on the tumours themselves. Obviously, a better understanding of the individual host-tumour relationship is going to be an essential feature for the design of chemotherapy best suited to the patients' individual needs but, unfortunately, we are still some way from this goal. In this paper, we shall examine a number of factors that may need to be borne in mind when attempting to devise new treatment strategies for the prolongation of useful life in patients with colo-rectal cancer.

TUMOUR GROWTH

The growth rate of colo-rectal cancer, as with any other cancer, is dependent upon the difference of the rate of cell production and the rate of cell loss. In the normal bowel, these factors are balanced and cell production is equal to cell loss, so that the crypt cell population remains constant. Normally, proliferation is restricted to the lower half of the crypts, but this spreads upwards throughout the length of the crypt and on to the flat epithelium between the crypts in response to acute and chronic injury [29, 30] and in adenomatous polyps, which are not necessarily pre-malignant; this abnormal distribution of proliferative cells persists and is a feature of those parts of the neoplastic glands that are in close proximity to the surface of the tumour in a position analogous to the upper part of a normal crypt.

Cell loss from a bowel tumour is by exfoliation both into the crypt and the lumen, the random loss of cells as a result of many different internal and external factors [8] and the migration of cells away from tumour sites *via* the lymphatics and blood stream. The latter, although these are very minor components in the daily cell loss, is the most important biologically for it ultimately is one of the factors controlling metastases, the other being the ability of such cells to proliferate and establish colonies in distant sites. The realization that many adenocarcinoma cells avidly phagocytose damaged adjacent tumour cells, has brought an explanation for many of the various inclusion bodies that have been described in colon cancer in the past and it is now very likely that they just represent a part of the recycling process of degraded material within the tumour [24].

The measurement of cell kinetics of human bowel cancer poses many technical as well as ethical problems. In a few instances percentage labelled mitosis (PLM) curves have been made after labelling with tritiated thymidine (^{3}H-TdR) *in vivo* which have indicated a cell cycle time of 24-28 hrs [30, 44]. Direct measurements of tumours using repeated double contrast barium enemas in patients with bowel cancer have been made by WELIN et al. [47]. In their study of 20 patients a mean tumour volume doubling time of 620 days was observed; obviously these patients represent a selected group in whom intestinal obstruction was not a complicating factor. Labelling of fragments of tumour with ^{3}H-TdR *in vitro* has been attempted by a number of authors [4, 28] who observed labelling indices of 9-24 %. We have repeated these experiments in our laboratory but found there was great variation, both from one tumour to another, and from one fragment to another, within the same tumour. This is probably an expression both of technological problems associated with tissue damage and irregular penetra-

tion of the isotope as well as a true biological variation within the tumour. On the other hand, the stathmokinetic technique in which mitosis is blocked in metaphase giving the patient colchicine or vincristine prior to surgery and calculating the movement of cells into mitosis [41] has yielded useful information. CAMPLEJOHN et al. [4] found the mean rate of entry of cells into mitosis/h was 0.5 %; in our laboratory, we observed a wide range from 0.9 % to 7.7 %. There are considerable variations both within groups of tumours of apparently the same degree of differentiation and these appear to be unrelated to the Dukes' groups and support the heterogeneity of ^{3}H-TdR labelling that was observed *in vitro*. By taking the reciprocal of the rate of entry of cells into mitosis the potential doubling time of tumours can be calculated to be between 159 and 144 hrs [4]. These authors also deduced from the available evidence the cell loss rate from bowel tumours must account for about 98 % cell production.

The successful development of the xenograft system for the growing of human cancer as transplants in immunodeprived mice has provided a fresh opportunity to study their growth kinetics. PICKARD et al. [40] examined colo-rectal xenografts in their first passage using the PLM technique, found that mean duration of G_2 was 6 hrs and the mean S was 14 hrs. However, it was found that it was very difficult to obtain precise kinetic data in this system. The doubling time increases progressively with the size of the tumour and the shape of the PLM curves strongly indicates a wide variation of the duration of G_1 phase which is typical of human tumours growing in man [44]. Furthermore, there was once again considerable variation in the behaviour of one tumour to another as well as in different transplants from the same tumour. The mean labelling indices in these tumours was 18.3 % with upper and lower limits of 27 % and 9 %. This is very comparable to those found in the transplantable colon cancer system that has been developed in mice by BALL and DOUBLE [1], whose labelling indices were 19 % to 24 %. All these data suggest that the growth fraction in human colon cancer may be higher than was originally suspected. Much of its chemoresistance may be bound to intrinsic properties of the cells and the heterogeneity of the clones making up a tumour, rather than a high proportion of cells in G_0, which has not been substantiated, at least in our model system, where it could be studied extensively.

Although the examination of primary tumours and xenografts of these tumours provides certain information about the behaviour of colonic cancer, it is the metastases that are the most important element in tumour growth, so far as the chemotherapist is concerned. The studies of CHARBIT et al. [6] have shown that metastatic adenocarcinomas from many different sites of origin when growing in the lung have a doubling time that is faster than their corresponding primaries. They calculate the mean doubling time to be 90 days, whilst that of primaries is 166 days. COLLINS [7] made a specific study for the behaviour of metastases of carcinoma of the colon and rectum and obtained a mean doubling time of about 100 days. These observations may be a reflection of a higher growth rate in metastatic cancer. On the other hand, it could be an artifact due to the study of these cancers growing in a site such as the lung, where the opportunity for the removal of necrotic tissue and mucin may be reduced compared to the primary site in the bowel. Finally in the consideration of the biology of tumour growth in relation to treatment, it is important to realize that colo-rectal tumours are very likely to follow a Gompertzian growth curve, which means that the rate of tumour growth is at its *maximum* when the tumour is smallest and diminishes progressively as the tumour increases in size. Indeed the experimental studies in the xenograft system appear to confirm this hypothesis [40], hence for the treatment of the tumour it would seem to be important for several reasons that chemotherapy should be given at a time when the tumour is minimal and the greatest number of cells are likely to be in the S phase, which is the most vulnerable part of the cell cycle.

ABNORMALITIES OF MUCOPOLYSACCHARIDES AND CELL PLASMA MEMBRANES

Although the detailed studies of the antigenicity of colon cancer cells and their associated oncofetal antigens have focused attention on the plasma membranes, relatively little work has been done on the mucopolysaccharides. Normal colonic mucosa is a highly active tissue for the synthesis of mucins. Recently, studies of the colon in rats and mice during the induction of tumours by dimethylhydrazine have shown that the mucopolysaccharides become abnormal and the kinetics and distribution of the enzymes controlling their synthesis and degradation are disturbed [35, 36]. FILIPE and COOKE [17] demonstrated an increase in both total hexosamines and sialic acid in human colonic tumours in the mucosa adjacent to adenocarcinoma; comparable results have been reported by BARKER et al. [2]. On the other hand, KIM et al. [25] found that galactosamine, glucosamine, fucose and sialic acid were reduced significantly in the tumour whereas galactose was reduced to a lesser extent and the mannose content remained unchanged. But they could not find any significant quantitative changes between the glycoside contents of the normal mucosa and the mucosa adjacent to tumours.

KIM et al. [25] also detected significant reduced activities of glycosyltransferases in the subcellular membrane fractions of human colon cancers. FILIPE and BRANFOOT [16] in a histochemical study have demonstrated that human colonic tumours and their adjacent mucosa show an abnormal increase of sialo-mucins and a reduction of sulphomucins. This effect may be detected in a patchy fashion at a considerable distance proximal to the tumour site. Modification of the lactic-dehydrogenase (LDH) isoenzyme patterns in the mucosa at a distance from a colo-rectal tumour is further evidence of a generalized field change into the bowel [20]. However its role, if any, in determining local recurrence is unknown.

ELECTRONMICROSCOPIC STUDIES OF COLONIC TUMOURS

Ultrastructural differences between rat colonic tumours, colonic epithelia of normal and tumour-bearing animals and foetal colon have been examined stereoscopically using image analysis technique (MIAN, personal communication). These studies indicated a significant decrease in the area of the luminal surface membrane of the tumour cell due to reduction in the size and density of microvilli compared with normal colonic cell. On the other hand, interdigitation of the lateral plasma membrane was increased in majority of the tumours (figs 1-4). Similar observations, but qualitative in nature, have been made previously on adenomatous polyps of the human colon by KAYE et al. [23] and ISMAI and STEIN [21]. The present study in rats, in contrast to those of the human tissue, indicated no significant quantitative difference in these parameters and in the general ultrastructure of the epithelial cells from different zones of the normal crypt of Lieberkühn. The ultrastructure of apparently normal epithelium of tumour-bearing animals appeared to be of an intermediate type between neoplastic and normal epithelia. In rats the epithelial cells of 18-day-old foetuses and 1-h-old newborns were intermediate between the normal and malignant cells but comparable with the cells from the mucosa of tumour-bearing animals in their ultrastructural features. These observations evidence some interesting points about the lack of similarities between the malignant colonic epithelium and its normal immature and foetal counterparts.

COLONIC TUMOUR ENZYMES

Apart from the enzymes directly associated with the glycosidic macromolecules other enzymes have been studied in the human colon cancer particularly to see if high levels of certain hydrolases might be exploited to liberate alkylating agents en-

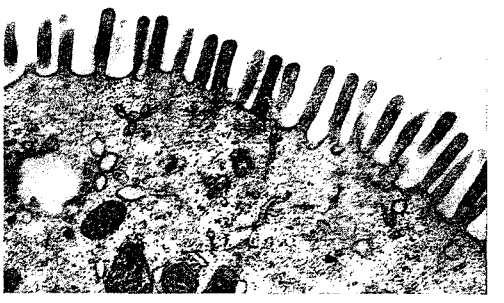

Fig. 1 - Microvilli of the colonic cells of a neonatal rat. Note the regular but short structure of the villi; x 33,280.

Fig. 2 - Microvilli of the cells at the upper part of the crypt of the colon in a normal adult rat. Note the well developed terminal web immediately below the villi; x 33,280.

Fig. 3 - Cell surface of the colonic cells in a rat in an area adjacent to a tumour induced by dimethylhydrazine. Note the reduction in height of the microvilli; x 33,280.

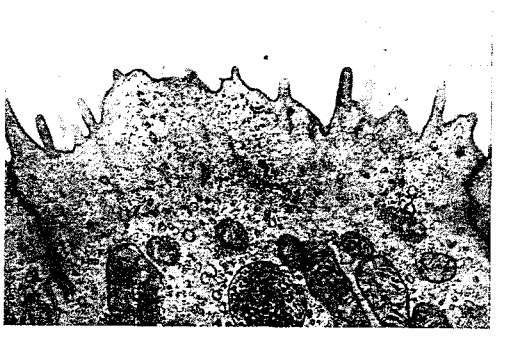

Fig. 4 - Cell surface of a colonic cancer induced in a rat by the injection of dimethylhydrazine. There is a great loss of microvilli and absence of the terminal web; x 33,280.

tering the cell conjugated to an appropriate cationic group. BALL and DOUBLE (personal communication) in our laboratory at Leeds have made a biochemical analysis of β-glucuronidase, acid and alkaline phosphatase and sulphatase in homogenates of colorectal cancers. They observed a widespread variation in these enzymes from one tumour to another and this was not correlated with the differentiation, Dukes' group or plasma CEA level. However, it is still possible that particular high values of an enzyme which could vary by as much as 10-fold could be the basis of drug selection. Histochemical studies of colon carcinomas and polyps, whether adenomatous or villous, have generally tended to indicate that the hydrolytic enzymes are reduced compared to normal bowel mucosa [26], 5'-nucleotidase being a marked exception. Interpretation of some of the biochemical assays may have to be taken with caution as some of the non-tumour cell elements may particularly be rich in one enzyme, e.g. macrophages contain large amounts of acid phosphatase. Studies of the oxyreductase enzymes, which reflect activity in the main pathways of intermediary metabolism, have been examined histochemically [27]. A higher activity was observed in the enzymes reflecting anabolism (NADPH-tetrazolium-reductase and enzymes depending on it), other oxyreductase enzymes showed far more variable activity.

These studies all point towards a considerable heterogeneity in the constitution of apparently similar tumours as judged by histological criteria. We are still uncertain of which if any of these particular expressions of biochemical constitution are important in determining characteristics such as invasion and antigenicity and susceptibility to chemotherapy that can profoundly influence the patients' outcome.

THE IMPROVEMENT OF SURVIVAL STATISTICS

The main problem for the clinician is how to arrest the deaths from colo-rectal cancer. At first it looked that the widespread introduction of the measurement of plasma CEA might be the basis of population screening, particularly for the more vulnerable age groups (60-80 years). However, this idea has lost favour as the CEA test has not been found to be specific for colo-rectal cancer [32, 38, 42] and it has been considered that its use as a general screening agent would lead to a large number of persons having to undergo extensive investigation to find whether an elevation of their CEA was an early sign of a cancer or associated with a far less dangerous cause. The fact that in the early cancers Dukes' classification A and B the % of patients with raised CEA levels has only been found to be 30 % and 54 % respectively [31, 32], is a further reason for not using CEA as a screening test for bowel cancer as it will be normal in about half those patients in which the surgeon is most anxious to diagnose as early as possible. At present it would seem that there is no substitute for a careful examination of the large intestine using double contrast barium enema, sigmoidoscopy and possibly colonoscopy when this is available.

At an even more elementary level the testing of the stools of any patient with an unexplained anaemia will often reveal the presence of chronic intestinal tract haemorrhage which may well originate from a neoplasm, tumours of the caecum and ascending colon are often symptom-free in their earlier stages. Finally, in view of the strong probability that many carcinomas of the large intestine in man originate from malignant transformation in polyps, whether adenomatous or villous, albeit this transformation may only occur in 1 or 2 %, it is clear that the removal of polyps is a cancer preventive measure and it is equally true that patients who have had a polyp constitute a higher risk group than the population in general. All patients who are in a high risk group should be offered the benefits of a regular follow-up examination and warned of the dangers of ignoring changes in bowel habit that may be the signs of an underlying neoplasm.

Nevertheless, there is considerable evidence to suggest that the preoperative CEA level can be a valuable guide to prognosis. Lo GERFO and HERTER [32] followed 150 patients for 24-36 months who had undergone 'curative' resections for colo-rectal cancer and showed that if their preoperative CEA value was raised there was a tendency to develop recurrence that was 1.8 times greater than in patients with a normal CEA at the time of resecting their primary cancer. Obviously this has great significance for the surgeon and we may have to alter his follow-up routine to take this into account, often all patients are discharged from follow-up too prematurely.

The second line of attack has been to make a greatly increased effort in the search for effective forms of chemotherapy, these have been greatly encouraged and supported by the National Cancer Institute. The current *status* of chemotherapy in colorectal cancer has been reviewed [5, 37]. Unfortunately, the overall picture is both disappointing and confused, this is partly due to the intrinsic insensitivity of colo-rectal cancer to chemotherapy and problems of drawing conclusions from trials made on patients at different stages of the disease and no doubt influenced by host-tumour interrelationships that are ill understood and profoundly alter the duration of life in the untreated patient, e.g. the extent of cachectic reactions, and anaemia can vary greatly for the same tumour load in different patients. There are a number of tactical moves that can be followed: a) treatment of established metastases; b) adjuvant chemotherapy after surgery; c) treatment of minimal residual disease; d) to monitor the patient (Dukes' A, B, C) and commence therapy when there is an indication that recurrence or metastasis has occurred. CARTER and FRIEDMAN [5] and MOERTEL [37] reviews have covered these first two areas, broadly speaking about 20 % of patients obtain objective remission in advanced disease, and the adjuvant programmes used so far are without effect. As yet there is little information on minimal residual disease, however there is growing evidence that postoperative monitoring can be a powerful technique to give the physician an earlier warning of metastases. MACKAY et al. [33] reported observations on serial CEA measurements in 220 patients with potentially curable colo-rectal cancers (fig. 5). In a 2-years follow-up 53 had developed recurrences or metastases. High rises in plasma CEA occurred synchronously or between 3 and 18 months before recurrences or metastases in 36 patients. LIVINGSTONE et al. [31] followed 53 patients with Dukes' A, B or C for 3 years. All of them have remained CEA-negative and tumour-free, other patients in their series showed rising CEA titres as the first event leading to the discovery of metastases and this has been the experience of others [3, 32, 33, 38, 40]. However, whilst serial observations of CEA can help with the earlier detection of metastases and a high rise could be an indication for chemotherapy, this test alone has its limitations. It may be negative at a time when metastases are present and it only gives information on one aspect of a very complex series of biochemical changes that can accompany the evolution of colo-rectal cancer. COOPER et al. [11] and STEELE et al. [45] have examined the role of serial serum enzyme determinations in monitoring colo-rectal cancer. They found that glutamyltranspeptidase, 5'-nucleotidase and alkaline phosphatase are useful parameters to measure in conjunction with CEA and indicate that there is probably a period of 3-9 months during the growth of liver metastases when the liver shows only a mild degree of biochemical abnormality as reflected in the rates of rise of these marker substances in the blood. This eventually leads into a final phase in which there is a more rapid and high rate of rise in the levels of these enzymes, although this phase can last for 1 year or more before the patient finally becomes jaundiced.

Evidence of host-tumour relationships is more difficult to establish, evidence from immunological studies is still fragmentary and as yet difficult to correlate with the patterns of evolution of the disease. We shall not discuss the immunological aspects of the disease in this article. However, there are disturbances of plasma proteins

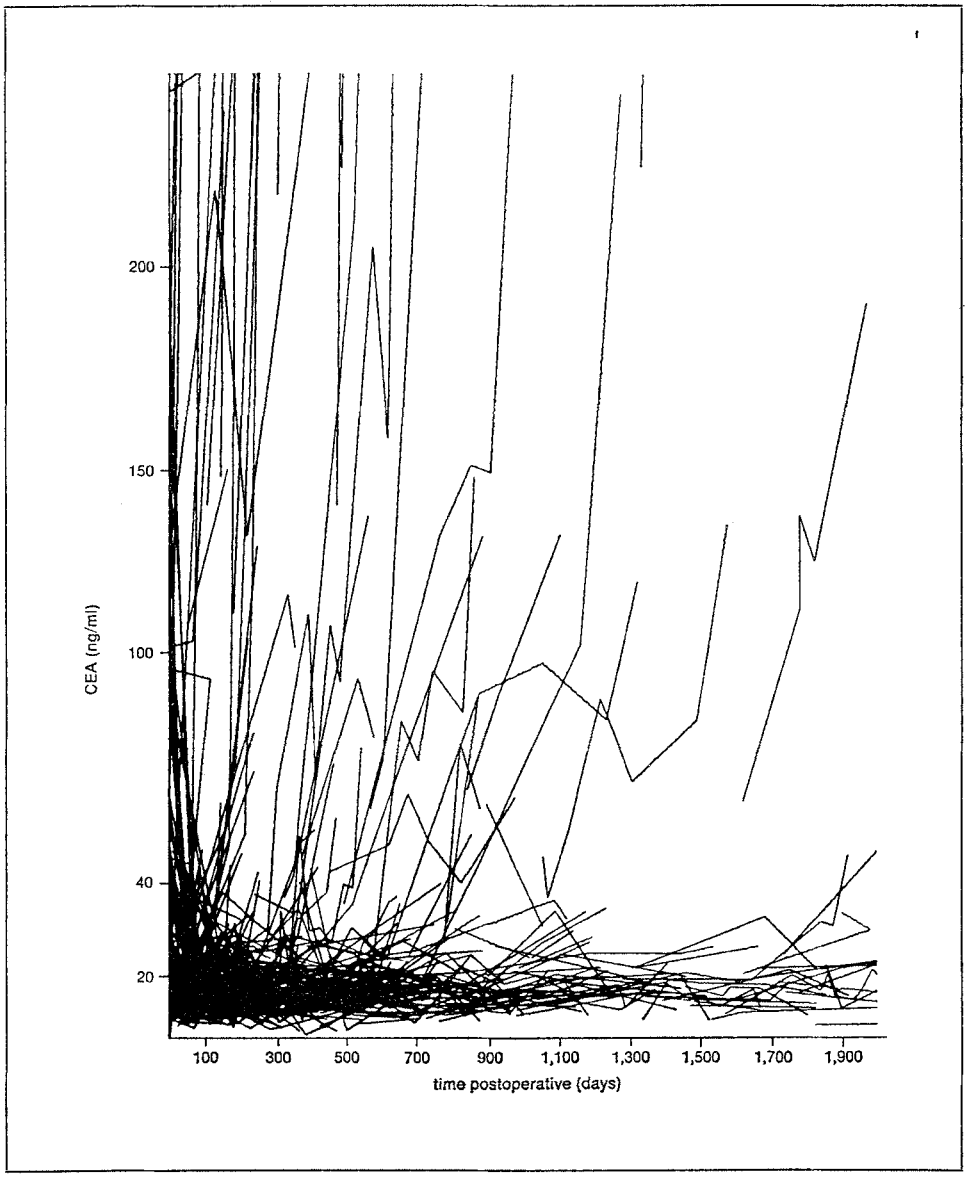

Fig. 5 - Computer printout of the serial estimations of plasma CEA (Todd assay) in 450 patients under study in Leeds. The assays were performed by Prof. A. Munro Neville, Chester Beatty Institute, London. The mean normal value is 12.5 ng/ml; from this series it is now considered that a postoperative rise above 30 ng/ml is suspicious of metastases. The rapidly rising values were found in patients with liver metastases.

that are being detected and no doubt their profiles will eventually lead to a better understanding of the way to classify patients following an apparently successful resection of a tumour. We need to know whether the patient is cured or whether he still has latent tumour foci that eventually will present as clinical metastases. In our laboratory we have measured changes in the α-globulins and observed α_1-globulins were raised in 44 % of patients with metastatic colo-rectal cancer, but in only 9 % of those with primary tumours. On the other hand, the α_2-globulin was raised in 88 % with primary tumours, in 59 % of postoperative patients with a normal CEA and GGT (γ-glutamyltranspeptidase), in 58 % of postoperative patients who were clinically normal but had an elevated CEA and 75 % with confirmed liver metastases. α_2-macroglobulin was not a significant component of the elevated α_2-globulins, but many of those patients with elevated α_2-globulins had an elevated haptoglobin [9]. This preliminary work suggests that in colo-rectal cancer study of the acute phase proteins may add new factors that could be used for monitoring; among the more promising groups of proteins for investigation in colo-rectal cancer are the α_2-haptoglobin [43] and the pregnancy-associated α_2-macroglobulin [46] and it is known that α_1-antitrypsin, α_1-glycoprotein and caeruloplasmin are liable to be elevated in cancer. Elevation of α-globulins is not correlated with the plasma CEA level [12] and we have confirmed this in our studies. There is possibly a link between the α-globulin levels and the immunosuppressive activity of the serum, a relationship that has been shown in many systems and in a small group of patients with colo-rectal cancer using the lymphocyte response to stimulation as the test system [19]. It will be interesting to see if an appropriate spectrum of serological measurements can be used to form the basis of assessing host-tumour relationships that may be helpful in selecting therapy. In this respect it has been found that serum IgM and IgA are raised in colon cancer being highest when there are distant metastases [34].

Furthermore, the serum muramidase (lysozyme) which reflects both the leucocyte count and the macrophage activity [18], can show an abnormally high level in primary colo-rectal cancer and in some patients with metastases [10]. Its response to metastatic cancer was unrelated to the level of the plasma CEA. COOPER et al. [10] interpreted this rise of muramidase as most likely reflecting alterations in the macrophage system as a response to the tumour. However, this interpretation has been challenged by JEDRZEJZAK and SIEKIERZYNSKI [22] who found that an elevated serum muramidase in cancer was often associated with a positive nitroblue-tetrazolium test [39] indicating an infection, and considered the rise of muramidase to be a secondary phenomenon, but the nitroblue-tetrazolium test itself is unreliable, which still leaves the matter an open question. This may turn out to be true, but in this context it is of interest to note that a much higher level of muramidase has been found in the sera of patients with Crohn's disease than in ulcerative colitis [15]. Obviously, more research is necessary to find out whether the muramidase level can help to describe the interaction of the host and the tumour.

Throughout this review we have drawn attention to the evidence that points repeatedly to the variability of colo-rectal cancer both in the constitution of the tumour itself and the responses it induces in the host. Undoubtedly some patients are cured, others respond well to chemotherapy, it seems vital to try to understand how they differ from those in whom the disease is more aggressive. It would seem desirable to concentrate the study on the time between surgery and the earliest signs of onset: detailed studies on the advanced hopeless cases can always produce a set of abnormalities to expose, but add very little to the knowledge, because the various inbalances in the homeostatic controls are so severe as to be beyond recovery.

SUMMARY

This article reviews the high incidence of colon cancer in Western countries; the contribution of cell biology and biochemistry to our knowledge of the behaviour of the disease is discussed. The use of a series of markers to obtain early evidence of metastasis and to make the prognosis is described. Some suggestions are made how to obtain improvements in the survival percentages.

REFERENCES

1) BALL C. R., DOUBLE J. A.: Transplantable Colon Tumours as Chemotherapy Screening Models - Cancer. (In Press).
2) BARKER S. A., STACEY M., TIPPER D. J.: Some Observations on Certain Mucoproteins Containing Sialic Acid - Nature (Lond.) *184*, 68, 1959.
3) BOOTH S. N., JAMIESON G. C., KING J. P. G., LEONARD J., DATES G. D., DYKES P. W.: Carcinoembryonic Antigen in the Management of Colo-Rectal Carcinoma - Brit. med. J. *4*, 183, 1973.
4) CAMPLEJOHN R. S., BONE G., AHERNE W.: Cell Proliferation in Rectal Carcinoma. A Stathmokinetic Study - Europ. J. Cancer *9*, 577, 1973.
5) CARTER S. K., FRIEDMAN M.: Integration of Chemotherapy into Combined Modality Treatment of Solid Tumors. II. Large Bowel Cancer - Cancer Treatment Rev. *1*, 111, 1974.
6) CHARBIT A., MALAISE E. P., TUBIANA M.: Relation between Pathological Nature and Growth Rate of Human Tumours - Europ. J. Cancer *7*, 307, 1971.
7) COLLINS V. P.: Time Occurrence of Pulmonary Metastases of Carcinoma of Colon and Rectum - Cancer *15*, 387, 1962.
8) COOPER E. H., BEDFORD A. J., KENNY T.: Cell Death in Normal and Malignant Tissues - Advanc. Cancer Res. *21*, 59, 1975.
9) COOPER E. H., TURNER R., GEEKIE A. D., GILES G. R., HALL R. R.: Variations in the Alpha Globulins in Colo-Rectal Cancer - Biomedicine. (In Press).
10) COOPER E. H., TURNER R., STEELE L., GOLIGHER J. C.: Blood Muramidase Activity in Colo-Rectal Cancer - Brit. J. Med. *4*, 662, 1974.
11) COOPER E. H., TURNER R., STEELE L., NEVILLE A. M., MACKAY A. M.: The Contribution of Serum Enzymes and Carcinoembryonic Antigen to the Early Diagnosis of Metastatic Colo-Rectal Cancer - Brit. J. Cancer *31*, 111, 1975.
12) CRAWLEY J. M., NORTHAN B. E., KING P. P. G., LEONARD J. C., BOOTH S. N., DYKES P. W.: The Effect of Serum Protein Concentration on the Specificity of the Radioimmunoassay of CEA in Malignant Neoplasm and Non-Malignant Disease - J. clin. Path. *27*, 271, 1975.
13) DOUBLE J. A., BALL C. R., COWEN P. N.: Transplantation of Adenocarcinomas of the Colon in Mice - J. nat. Cancer Inst. *54*, 271, 1975.
14) DUKES C. E.: The Classification of Cancer of the Rectum - J. Path. Bact. *35*, 323, 1932.
15) FALCHUK K. R., PERROTTO J. L., ISSELBACHER K. J.: Serum Lysozyme in Crohn's Disease and Ulcerative Colitis - New Engl. J. Med. *292*, 395, 1975.
16) FILIPE M. I., BRANFOOT B. M.: Abnormal Patterns of Mucous Secretion in Apparently Normal Mucosa of Large Intestine with Carcinoma - Cancer *34*, 282, 1974.
17) FILIPE M. I., COOKE K. B.: Changes in the Composition of Mucin in the Mucosa Adjacent to Carcinoma of the Colon as Compared with the Normal. A Biochemical Investigation - J. clin. Path. *27*, 315, 1974.
18) HANSEN W. E.: Plasma Lysozyme. A Measure of Neutrophil Turnover. An Analytical Review - Series Haemat. *7*, 1, 1974.
19) HSU C. C. S., LO GERFO P.: Correlation between Serum Alpha-Globulin and Plasma Inhibitory Effect on PHA-Stimulated Lymphocytes in Colon Cancer Patients - Proc. Soc. exp. Biol. (N.Y.) *139*, 573, 1972.
20) HUNT P. S., DENNIS P. M., JABLONSKI P., KHOO S. H., MCLEOD L., THOMSON H. D. P., WATTS J. M.: Studies of Lactic Dehydrogenase Content of Rectal Biopsies - Gut *15*, 972, 1974.
21) ISMAI H., STEIN A. A.: Ultrastructure of Adenocarcinoma of the Colon - Gastroenterology *44*, 410, 1963.
22) JEDRZEJZAK W. W., SIEKIERZYNSKI M.: Serum Muramidase Activity in Untreated Cancer - Brit. med. J. *1*, 735, 1975.
23) KAYE G. I., FENOGLIO C. M., PASCAL R. R., LANE N.: Comparative Electron Microscopic Features of Normal, Hyperplastic and Adenomatous Colonic Epithelium - Gastroenterology *64*, 926, 1973.
24) KERR J. F. R., WILLIE A. H., CURRIE A. R.: Apoptosis: A Basic Biological Phenomenon with Wide Ranging Implications in Tissue Kinetics - Brit. J. Cancer *26*, 239, 1972.

25) KIM Y. S., ISAACHS R., PERDOMO J. M.: Alterations of the Membrane Glycoproteins in Human Colonic Adenocarcinoma - Proc. nat. Acad. Sci. (Wash.) *71*, 4869, 1974.
26) KOUDSTAAL J., MAKKINK B., OVERDIEP S. H.: Enzyme Histochemical Pattern in Human Tumours. I. Hydrolytic Enzymes in Carcinoma of the Colon and Breast - Europ. J. Cancer *11*, 105, 1975.
27) KOUDSTAAL J., MAKKINK B., OVERDIEP S. H.: Enzyme Histochemical Pattern in Human Tumours. II. Oxidoreductases in Carcinoma of Colon and Breast - Europ. J. Cancer *11*, 111, 1975.
28) LIEB L. M., LISCO M.: *In Vitro* Uptake of Tritiated Thymidine by Carcinoma of the Human Colon - Cancer Res. *26*, 733, 1966.
29) LIPKIN M.: Phase 1 and Phase 2 Proliferative Lesions of Colonic Cancer - Cancer *34*, 878, 1974.
30) LIPKIN M., BELL B., STALDER C., TRONGALE F.: The Development of Abnormalities of Growth in the Colonic Epithelial Cells in Man - In: BURDETTE W. (Ed.): Carcinoma of the Colon and Antecedent Epithelium. Thomas, Springfield, 1970.
31) LIVINGSTONE A. S., RAMPSON G. L., SHUSTER J., GOLD P., HINCHEY J.: Carcinoembryonic Antigen in the Diagnosis and Management of Colo-Rectal Carcinoma - Arch. Surg. *109*, 259, 1974.
32) LO GERFO P., HERTER F. P.: Carcinoembryonic Antigen and Prognosis in Patients with Colon Cancer - Ann. Surg. *181*, 81, 1975.
33) MACKAY A. M., PATEL S., CARTER S., STEVENS U., LAURENCE D. J. R., COOPER E. H., NEVILLE A. M.: The Role of Serial Plasma CEA Assays in the Detection of Recurrent and Metastatic Colo-Rectal Carcinomas - Brit. med. J. *4*, 382, 1974.
34) MANDER A. M., BOLTON P. M., WHITEHEAD R. H., NEWCOMBE R. G., HUGHES L. E.: Immune Competence in Colon Cancer; Relation of Pretreatment Tests to Diagnosis and Tumour Stage - Brit. J. Cancer. (In Press).
35) MIAN N., COWEN D. M.: Glycosidases in Normal and Dimethylhydrazine-Treated Rats and Mice with Special Reference to the Colonic Tumours - Brit. J. Cancer *29*, 438, 1974.
36) MIAN N., COWEN D. M., NUTMAN C. A.: Glycosidase Heterogeneity among Dimethylhydrazine-Induced Rat Colonic Tumours - Brit. J. Cancer *30*, 231, 1974.
37) MOERTEL C. G.: Cancer of the Large Bowel - In: FREI E., HOLLAND J. F. (Eds): Cancer Medicine. Lea and Febiger, Philadelphia, 1973.
38) NEVILLE A. M., LAURENCE D. J. R.: Report of the Workshop on the Carcinoembryonic Antigen (CEA): the Present Position and Proposal for Future Investigation - Int. J. Cancer *14*, 1, 1974.
39) PARK B. H., FIKRIG S. M., SMITHWICK E. M.: Infection and Nitroblue Tetrazolium Reduction by Neutrophils - Lancet *2*, 532, 1968.
40) PICKARD R. G., COBB L. M., STEEL G. G.: The Growth Kinetics of Xenografts of Human Colo-Rectal Tumours in Immune Deprived Mice - Brit. J. Cancer *31*, 36, 1975.
41) REFSUM S. B., BERDAL P.: Cell Loss in Malignant Tumours in Man - Europ. J. Cancer *3*, 235, 1967.
42) REYNOSO G.: Carcinoembryonic Antigen in Patients with Different Cancers - J. Amer. med. Ass. *220*, 361, 1972.
43) RIMBAUT C.: L'α_2H-globuline-glycoprotéine réactionnelle sérique d'origine hépatique: ses rapports avec les affections malignes - Bull. Cancer *60*, 411, 1973.
44) STEEL G. G.: The Cell Cycle in Tumours: An Examination of Data Gained by the Technique of Labelled Mitoses - Cell Tiss. Kinet. *5*, 87, 1972.
45) STEELE L., COOPER E. H., MACKAY A. M., LOSOWSKY M. S., GOLIGHER J. C.: Combination of Carcinoembryonic Antigen and Gamma-Glutamyl Transpeptidase in the Study of the Evolution of Colo-Rectal Cancer - Brit. J. Cancer *30*, 319, 1974.
46) STIMSON W. H.: Correlation of the Blood Level of Pregnancy-Associated Macroglobulin with the Clinical Course of Cancer Patients - Lancet *2*, 777, 1975.
47) WELIN J., YOUKER J., SPRATT J. S.: The Rates and Patterns of 375 Tumors of the Large Intestine and Rectum Observed Serially by Double Contrast Enema Study (Malmö Technique) - Amer. J. Roentgenol. *90*, 673, 1963.

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