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THE INFLUENCE OF METABOLIC PHENOTYPES UPON THE DEVELOPMENT OF COLORECTAL NEOPLASIA

by

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CHAPTER 7 CONCLUSIONS

A major strength of this study was the selection of the control group from patients with a normal barium enema x-ray or colonoscopy. Thus, it is most unlikely that the control group would have included patients with asymptomatic adenomas or carcinomas. By using a different control group, a study might simply determine risk factors for colonoscopy and not for colonic neoplasia.

On the other hand, a potential drawback of our control group is that the individuals were not totally healthy and asymptomatic, as indicated by their need for these lower GI investigations. They are hospital-based controls and the observed associations may therefore not be true for totally asymptomatic community-based controls. For example, one possibility relevant to this study is that hospital-based controls may have a lower percentage of fast acetylators than community-based controls.

Several studies have suggested that acetylator phenotype has a role in the aetiology of colonic carcinomas (Ilett *et al.*, 1987; Lang, Chu and Hunter, 1986). They suggest that the fast acetylator is more predisposed to develop colorectal carcinoma than the slow acetylator. This is supported by this study which showed a significantly higher percentage of fast acetylators in those with colorectal carcinomas(42.9%) than in controls(30%). However, the strength of this observation is reduced below the significant level of $p<0.05$ if the Yates continuity correction is applied to the results of the Chi squared tests. There was also a study from Spain(Ladero, Gonzalez and Benitez, 1991) which showed no significant relationship between acetylator status and colorectal carcinoma. Nevertheless, when data from all studies were pooled, the association between colorectal carcinoma and the fast acetylator phenotype is highly significant($p<0.001$). However, the power of individual studies to detect differences in acetylator phenotype was relatively low and this issue may only be resolved by a large

multicentre study.

Dietary data were collected by a self-administered retrospective dietary food frequency questionnaire. There are some problems with the method including poor recall of dietary habits and recent chances in diet in an attempt to ameliorate gastrointestinal symptoms. The odds ratio of fast to slow acetylators between the normal and cancer patients is higher in the high meat consumer group than in the low meat consumer group of patients. Therefore, there may be an effect of meat consumption among fast acetylators which is more pronounced in females. However, caution must be exercised in this interpretation as the numbers of patients studied here was small. Further studies are needed to confirm and explain the observed gender differences. This study is consistent with the hypothesis that the combination of fast acetylator phenotype with a high consumption of meat substantially increases the risk of colonic carcinomas. It supports Lang's paper showing that the amount of food-borne heterocyclic amine carcinogens may have a different risk potential for colonic neoplasia between slow and fast acetylators (Lang *et. al.*, 1994).

Previous studies have not explored the relationship of acetylator status with colonic adenomas. This study showed that patients with colonic adenomas have a frequency of fast acetylators(32.6%) intermediate between controls and those with carcinoma. It therefore appears that there could be an association between fast acetylators and increased cellular proliferation, consistent with the colonic adenoma-carcinoma sequence.

In this study, the polymorphic distribution of NAT activity in the colonic tissue corresponded to the systemic acetylator phenotype. Thus, sulphamethazine acetylation in colonic tissue was significantly higher in fast acetylators than in slow acetylators. This indicates that acetylator phenotype can be expressed in colonic tissue as well as in hepatic tissue although polymorphic NAT activity only accounts for a minority of NAT activity. At present, the substrate specificity

of carcinogens for the two forms of NAT remain unclear.

A method of genotyping individuals for their acetylator status has been devised in this study. It is based on amplification of part of the NAT gene by PCR and subsequent digestion with restriction endonucleases, to ascertain the presence or otherwise of different NAT restriction sites that are unique to the different NAT alleles. This study demonstrates good concordance between biochemical phenotyping and genotyping for the acetylator phenotype. It also demonstrates that there is a higher frequency of the fast allele(F1) in those with colorectal carcinoma than in controls, although the numbers studied are too small to achieve statistical significance. The ability to determine acetylator genotype from leucocyte DNA in blood should facilitate further epidemiological studies to assess the role of acetylator genotype in various diseases including colon cancer.

This study corroborated previous reports of higher GST activity in colonic cancer. Furthermore, concentrations of tissue GSH were significantly higher in adenomas and cancer. The reasons for these changes remain unclear but they may account, in part, for the resistance of colonic neoplasms to chemotherapeutic drugs which are effective in other cancers.

As expected, colonic adenomas and carcinomas showed significantly higher cell proliferation than uninvolved mucosa. However, correlations of proliferative activity with GST activity, GSH concentration and NAT activity in the normal and carcinoma tissues and with acetylator phenotype were not statistically significant. Furthermore, there was no significant difference in the proliferative index(PI), as measured by flow cytometry, in the uninvolved mucosa of normal controls when compared to "normal" mucosa associated with adenomas or carcinomas, indicating that this measurement of PI cannot be used as a biomarker of risk for the development of colonic neoplasia. The PI and total GST were significantly higher in GSTμ null phenotype individuals than in GSTμ positive individuals in the carcinoma tissue. This maybe a chance

finding due to the small numbers studied since individuals of the GSTμ null phenotype were not at higher risk for the development of colonic neoplasia.