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High Levels of DNA Adducts in Human Colon Are Associated with Colorectal Cancer[1]

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ABSTRACT

Colon cancer is one of the most frequent causes of cancer death in western countries. Epidemiological studies suggest that colorectal cancer can be attributed, at least in part, to carcinogens and mutagens present in the diet and/or the environment. The covalent binding of these xenobiotics or their reactive metabolites to DNA is believed to initiate this chemical carcinogenesis. In the present study, using a ^{32}P-postlabeling method, we investigated DNA adduct levels in control colons from patients without colorectal adenocarcinoma and in nontumoral and tumoral tissues from patients with colorectal adenocarcinoma. Our results show that the DNA adduct level is significantly higher ($P < 0.001$) in nontumoral than in control or tumoral colon samples. For the first time, we demonstrate in humans that the presence of numerous adducts in colonic mucosa is associated with colorectal cancer, a finding in agreement with the importance of chemical factors in causing this disease; therefore, after confirmation of the link between DNA adducts and colorectal cancer, the measurement of DNA adduct levels in colon samples could constitute a useful approach to the early detection of colorectal cancer.

INTRODUCTION

Colon cancer is one of the most frequent causes of cancer death in developed countries (1). Both hereditary and, mainly, environmental factors (potential carcinogens and mutagens present in the diet and tobacco and alcohol consumption) contribute to the development of colorectal cancer (2–5). The process of chemical carcinogenesis is initiated by the covalent binding of carcinogens or their reactive metabolites to DNA, thus forming DNA adducts (6, 7). The presence of such adducts was demonstrated recently in human colonic mucosa (8). These adducts can be removed by DNA repair processes or by cell death, but they reach steady-state levels in carcinogen target tissues, reflecting the balance between exposure to carcinogens, adduct formation, and adduct elimination. DNA adducts can result directly in mutational events, leading potentially to cancer (9–11). These mutations in genes implicated in the control of cell growth lead to the formation of small benign tumors (adenoma), which may grow and may result finally in malignant tumors (carcinoma) (12). DNA carcinogen adducts in target tissues during chronic exposure seem to be dose related and could be predictive of human disease risk (13–15). The liver is the most active organ in metabolizing xenobiotics into active metabolites. These metabolites then can be conjugated, excreted in bile, and liberated in the colon, and after activation by acetyltransferases, for instance, they may react with DNA (16, 17). Moreover, the presence of carcinogen-activating enzymes also has been demonstrated in epithelium colon cells (18) and in colonic bacteria (19); thus, the production of reactive metabolites *in situ* in colonic mucosa is quite possible.

Therefore, we sought to determine DNA adduct levels in control colons from patients without colorectal adenocarcinoma and in nontumoral and tumoral tissues from patients with colorectal adenocarcinoma. A potential difference between colonic mucosa from affected (nontumoral tissue) and unaffected patients (sigmoiditis) could be in good agreement with the chemical origin of colorectal cancer. Moreover, we compared nontumoral and tumoral tissues.

We showed that both the quantity and variety of DNA adducts were the highest in nontumoral tissues compared with tumoral tissues and, more interestingly, to mucosa from control patients. The relevance of these results to tumor biology, and to early detection of colorectal cancer, is discussed.

MATERIALS AND METHODS

Chemicals. Proteinase K, apyrase, and RNases A and T1 were purchased from Sigma Chemical Co. (St. Louis, MO); T4 polynucleotide kinase was purchased from PL Biochemicals (Milwaukee, WI); micrococcal nuclease and spleen phosphodiesterase were from Worthington Biochemicals (Freehold, NJ); nuclease P1 was from from Boehringer Mannheim (Mannheim, Germany); [γ-^{32}P]ATP (5000 Ci/mmol) was from Amersham (Buckinghamshire, United Kingdom); 5′-deoxynucleotide was from Sigma; phenol was from Appligene (Illkirch, France); polyethyleneimine was from Corcat (Portsmouth, VA); and cellulose sheet MN 301 was from Machery Nagel (Düren, Germany).

Human Tissues. Human tissue samples were obtained, in accordance with French regulations and approval by the ethical committee, from the Department of Surgery of Hôpital Laënnec (patients undergoing surgery for sigmoiditis and colorectal adenocarcinomas) and from the Hôpital Broussais (Paris, France; control colons from organ donors). Clinical data from patients are given in Table 1. Eight control colon specimens were obtained from accidentally deceased organ donors (6 males and 2 females) ages 23 ± 14 years. Twenty-one control colon specimens were obtained from individuals (11 males and 10 females), ages 63 ± 16 years, after resection of part of the organ following cured sigmoiditis. Control tissues were taken from these resected organs in regions without any macroscopic lesions. Tumoral and nontumoral tissues were obtained from 18 patients (8 males and 10 females) ages 66 ± 13 years who underwent surgery for colorectal adenocarcinomas. Nontumoral specimens were taken from a macroscopically healthy region of the resected pieces at least 5 cm away from the tumor. Tumoral tisues were dissected out from the middle of the tumor; the tumoral region was macroscopically visible. Part of each tissue sample was reserved for histopathological examination, and the remainder was used for this investigation.

^{32}P Postlabeling of DNA Adducts. DNA from surgically removed control, nontumoral, and tumoral colons was extracted and purified as described previously (20). Purified DNA (6 μg) was hydrolyzed enzymatically in 3′-nucleotides before 5′-^{32}P phosphorylation by [^{32}P]ATP and T4 polynucleotide kinase according to the method described by Reddy and Randerath (21) and modified by Pfohl-Leszkowicz *et al.* (22). Adducts were separated by chromatography on polyethyleneimine cellulose plates. Quantification was performed by Cerenkov counting of individual spots, and the results were expressed after substraction of blank values.

Statistical Analysis. The level of significance was tested between control and nontumoral tissues by the distribution-free Mann and Whitney unpaired *t*

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Table 1 *Clinical data of the patients investigated*

Patient	Gender	Age (yr)	Diagnosis	Dukes	Astler-Coller	Tumor differentiation
Normal colons						
15	F[a]	41	Traffic accident, kidney donor			
16	F	11	Traffic accident, kidney donor			
20	M	48	Traffic accident, kidney donor			
21	M	16	Traffic accident, kidney donor			
24	M	15	Traffic accident, kidney donor			
32	M	15	Traffic accident, kidney donor			
34	M	19	Traffic accident, kidney donor			
41	M	22	Traffic accident, kidney donor			
Normal colons (sigmoiditis)						
128 S	F	54	Sigmoiditis			
130 S	M	47	Benign polyps			
131 S	M	19	Sigmoiditis			
132 S	M	63	Sigmoiditis			
139 S	M	69	Sigmoiditis			
141 S	M	48	Sigmoiditis			
142 S	F	57	Sigmoiditis			
143 S	F	75	Sigmoiditis			
144 S	M	47	Sigmoiditis			
146 S	F	80	Sigmoiditis			
147 S	F	81	Sigmoiditis			
151 S	M	78	Sigmoiditis			
153 S	M	76	Sigmoiditis			
157 S	F	85	Sigmoiditis			
159 S	F	73	Sigmoiditis			
160 S	F	56	Sigmoiditis			
165 S	M	63	Sigmoiditis			
169 S	F	72	Sigmoiditis			
171 S	M	54	Sigmoiditis			
175 S	F	49	Sigmoiditis			
176 S	M	73	Sigmoiditis			
Tumoral and nontumoral colons						
124 T and NT	M	57	Sigmoid adenocarcinoma	A	A	Well differentiated
125 T and NT	F	43	Colon adenocarcinoma	B	B2	Well differentiated
133 T and NT	F	80	Sigmoid adenocarcinoma	A	B1	Well differentiated
134 T and NT	M	77	Sigmoid adenocarcinoma	A	B1	Well differentiated
135 T and NT	F	70	Right colon adenocarcinoma	C	C1	Moderately differentiated
138 T and NT	M	75	Sigmoid adenocarcinoma	C	C1	Moderately differentiated
140 T and NT	M	65	Sigmoid adenocarcinoma	C	C1	Well differentiated
145 NT	F	48	Colorectal adenocarcinoma	B	B2	Well differentiated
148 T and NT	F	66	Right colon adenocarcinoma	B	B2	Moderately differentiated
150 T and NT	M	72	Left colon adenocarcinoma	C	C1	Well differentiated
155 T and NT	F	54	Sigmoid adenocarcinoma	D	D	Well differentiated
158 T and NT	F	83	Rectum adenocarcinoma	C	C2	Well differentiated
162 T and NT	M	81	Left colon adenocarcinoma	B	B2	Well differentiated
163 T and NT	F	64	Right colon adenocarcinoma	C	C1	Well differentiated
166 T and NT	M	77	Rectosigmoid adenocarcinoma	C	C1	Well differentiated
170 T	M	56	Sigmoid adenocarcinoma	D	D	Well differentiated
172 T and NT	F	44	Right colon adenocarcinoma	C	C1	Well differentiated
173 T	F	80	Left colon adenocarcinoma	C	C2	Moderately differentiated

[a] F, female; M, male; S, control colons from patients with cured sigmoiditis; NT, nontumoral; T, tumoral colons from patients with colorectal adenocarcinoma.

test and between nontumoral and tumoral tissues by the distribution-free Wilcoxon paired *t* test (23).

RESULTS AND DISCUSSION

There is considerable evidence demonstrating the genotoxic activity of numerous compounds present in the diet and in tobacco. These compounds include mycotoxins, plant alkaloids, food additives, pesticides, polycyclic aromatic hydrocarbons, and heterocyclic amines present in tobacco smoke and cooked fish and meat (3–5). The gastrointestinal tract is the first organ exposed, but these compounds seem to exert their deleterious effects exclusively in the colon. Indeed, the incidence of cancer of the small intestine is 40–60 times lower than that of colon (24), and this difference is in good agreement with the results of Hamada *et al.* (25), who showed, in a Japanese population with a mean age of 67 years, that the total level of DNA adducts in the small intestine was 28-fold lower than in the colon.

DNA adduct detection was carried out by the very sensitive ^{32}P-postlabeling method on several types of colon samples from different populations: organ donors (control colons), patients with cured sigmoiditis (sigmoiditis control colons), and patients with colorectal adenocarcinoma (nontumoral and tumoral colons). Quantification of DNA adducts in control, nontumoral, and tumoral colon samples is presented in Table 2, and representative profiles of DNA adducts in each kind of tissue are shown in Fig. 1. No DNA adduct in control colons from organ donors (detection limit, 1 adduct/10^{10} nucleotides) was detected, whereas a few adducts were observed in control colons from patients operated on after cured sigmoiditis. Yet, the DNA adduct level remained low; it was even undetectable in 4 of the 21 patients (sigmoiditis control colons) studied. Among the colon samples studied, 24 different adducts were detected; adducts 17–24 were so extremely rare and weak that they are not presented in either Table 2 or Fig. 1. The interindividual differences in the DNA adduct levels were very great. The adduct levels ranged from 0.5 to 328.5 adducts/10^9 nucleotides. In most of the DNA samples that were positive in the ^{32}P-postlabeling method, a dominant spot was detected, which ranged from 0.5 to 127 adducts/10^9 nucleotides (adduct 1). Our result is in accordance with a recent published study (8) showing the presence of a mucosa-specific DNA adduct in all adult colorectal samples studied. On the other hand, some of these adducts were detectable only in samples from patients with colorectal cancer (adducts 6, 13, and 14).

Table 2 *Quantification of DNA adducts in human control, nontumoral, and tumoral colorectal tissues*[a]

Colon samples	Adduct																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
128 S[b]	4.7	0.8	3.1	0.8	1			0.9									11.3
130 S	0.5								0.5		0.4						1.4
131 S	0.4		0.4														0.8
132 S	1.75		1.15	0.85													3.75
139 S	6.4		2	0.4			0.55										9.35
141 S	1.6																1.6
142 S																	0
143 S	3.2	1.6	2.1							2.3	1.8						11.00
144 S	1.1											0.9					2
146 S	0.9										1.1						2
147 S																	0
151 S			5.3	1.7	1.1		0.95										9.05
153 S																	0
157 S																	0
159 S	1.6		1.7		1.3						1.7	1.9			1.2	2.9	12.3
160 S	2.7		2.9		2.2				1.8		1.7	2.2			1.6	8.2	23.3
165 S	0.6																0.6
169 S	2.3	0.5	1.25					0.6			1.15						5.8
171 S	0.4																0.4
175 S	2.3																2.3
176 S	0.8										0.5						1.3
124 NT	46.1	6.25	13.8	5.2	4.6	9.4	4.7					3.9	3.2				97.15
125 NT	11.8		16.4		3				11.9		1.15					1.8	46.05
133 NT	4			0.5	3.6			1.4	1.1			1	0.62			2	14.22
134 NT																	0
135 NT	17.1	1.9		15.3													34.3
138 NT	0.6								0.2		0.3						1.1
140 NT	62.8	7.5	14.1	19.7	17.3	6.5	6.7						4.5			5.4	144.5
145 NT	126.8	11.7	19.5	12.5	13.9	10.7	6					4		5.5		16	226.6
148 NT	1.5	0.4										0.4					2.3
150 NT	0.5																0.5
155 NT	39.2	15	55.8		33.2		33			18.1	45.4	59.8		14.7		14.3	328.5
158 NT	15.2			30	28.4			3.3	4.6	24.6	14.7					20	140.8
162 NT	13.6	12.1		19	17.8				2.1		12.5					19.7	96.8
163 NT	11.6															15.8	27.4
166 NT	55	17.2								9	18.1		0.8				100.1
172 NT	81.2	11.9	39	12.9	14.2	16.5						9.3	7.7			7.8	200.5
124 T	8.5	1.3	4.3	1.5	2.4		1.5			2.2			1.5				23.2
125 T	2.5									1.55	1.41	1.55					7.01
133 T				0.6	1.4			0.5	1.2				2.3			1.1	7.1
134 T																	0
135 T	0.7								0.6		0.65						1.95
138 T																	0
140 T	4.6	0.4	4.4	1.3	2.8					0.8		0.6					14.9
148 T	0.5											0.4					0.9
150 T																	0
155 T	1.6		1	0.9								0.95					4.45
158 T	1.6			1.4												2	5
162 T	2			1.4													3.4
163 T	1.2																1.2
166 T	1.6	1.1			3.7								1				7.4
170 T	0.3																0.3
172 T	10	1.4	7.5	2.5	1.3	1.3	1.1			1.1		1					27.2
173 T	0.5																0.5

[a] Results are expressed as number of adducts/10^9 nucleotides.
[b] S, control colons from patients with cured sigmoiditis; NT, nontumoral; T, tumoral colons from patients with colorectal adenocarcinoma.

The average level of DNA adducts calculated for each group revealed that the highest amount of DNA adducts was observed in nontumoral samples (Fig. 2*A*). Between control (sigmoiditis; 4.7 ± 6.0 adducts/10^9 nucleotides) and nontumoral (91.3 ± 95.6 adducts/10^9 nucleotides) colon samples, this difference was highly significant ($P < 0.001$ by the Mann and Whitney t test). In control colon samples, no more than 25 adducts/10^9 nucleotides were detected, whereas in nontumoral colon samples, 11 of 16 patients (69%) presented more than 25 adducts/10^9 nucleotides (Fig. 2*B*). These differences were even more striking when compared with the organ donor population.

Finally, the mean level of DNA adducts from tumoral tissues (6.15 ± 8.2 adducts/10^9 nucleotides) was significantly lower ($P < 0.002$ by the Wilcoxon paired t test) compared with nontumoral tissue from the same patient (Fig. 3). Differences in DNA adduct levels between nontumoral and tumoral samples also have been reported to occur in human lung tumor tissues compared with adjacent nonmalignant tissues (26). This could be due to lower adduct formation. Indeed, in a previous study (27), we showed several changes in drug-metabolizing enzymes in colorectal tumoral tissues compared with nontumoral tissues. In tumoral tissues, a significant decrease in cytochrome P450 3A, implicated in the metabolism of numerous carcinogens, such as aflatoxin (28), and an increase in glutathione transferase resulted in lower formation of electrophilic metabolites and, consequently, a lower formation of adducts. This also may have been due to faster adduct elimination. Adducts may be diluted by more rapid cell proliferation or aberrant DNA repair in the tumor.

Given the rapid cell turnover from 3 to 8 days in the human colon (29), the substances forming DNA adducts would seem to originate mainly from recently consumed food, alcohol, and/or tobacco. Observed differences from patient to patient could reflect: (*a*) variations in food composition and, therefore, variations in genotoxic com-

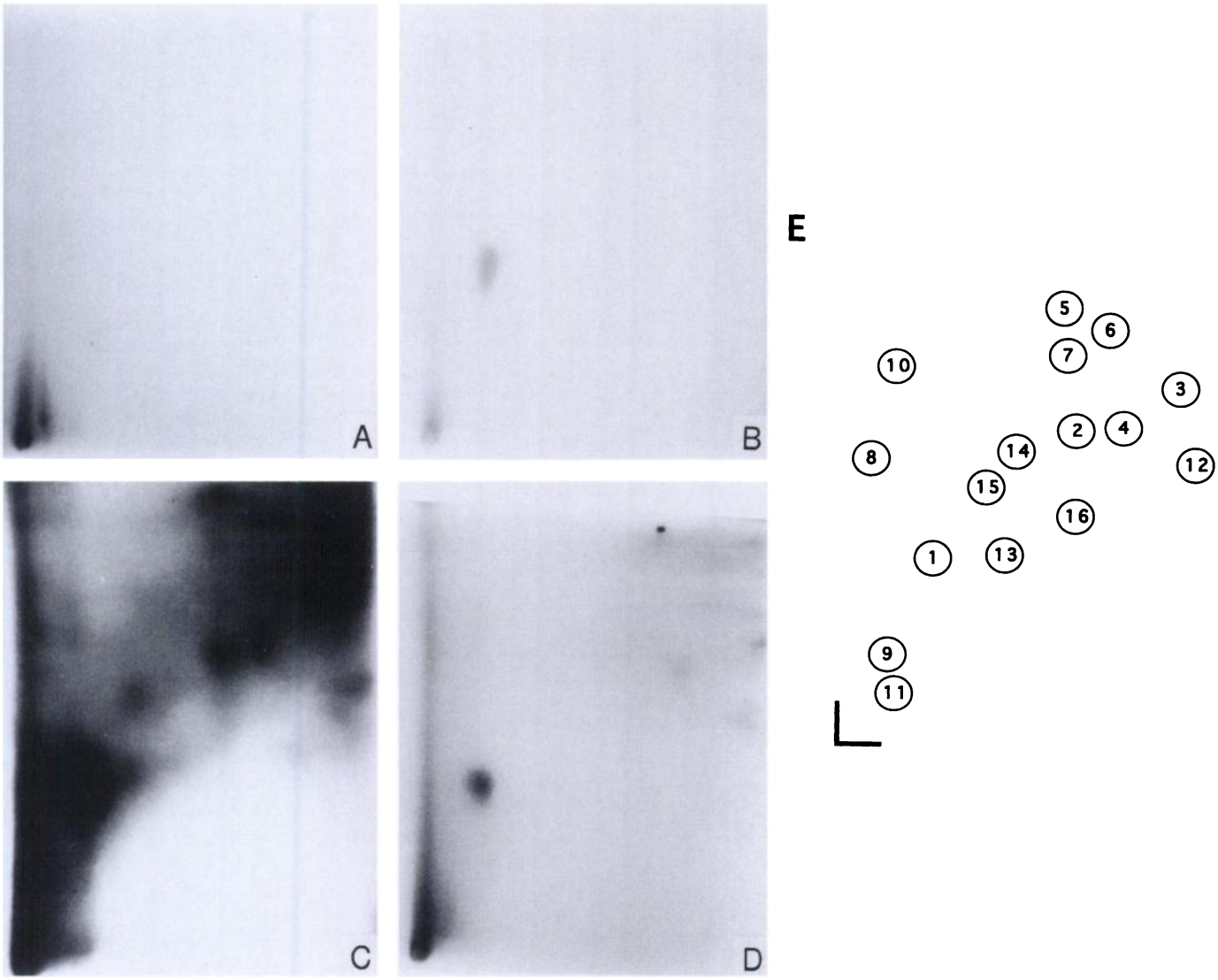

Fig. 1. Autoradiography of DNA adducts in human colon samples. Exposure time of 3 days for each sample. *A*, control colon from organ donor; *B*, control colon from patient with cured sigmoiditis; *C*, nontumoral colon; *D*, tumoral colon from the same patient with colorectal adenocarcinoma; *E*, corresponding mapping. *Numbers* correspond to adduct numbers in Table 2.

pounds to which these patients were exposed; (*b*) genetic or environmental variations in xenobiotic-metabolizing enzymes; or (*c*) the rate of elimination of these adducts. It is, therefore, interesting to observe that in the nontumoral tissues from patients with colon cancer, the adduct level is significantly higher than in every other tested category. This would mean that these individuals either have had strong exposure to genotoxic compounds, a higher capacity to bioactive them, and/or a lower capacity to detoxicate them, and/or that they have a slower rate of elimination of these adducts. It also can be hypothesized that the exposure remained similar between the time of initiation (several years ago) and the time of the adduct measurement.

It was very difficult to find an ideal control population. Indeed, this population should match for age and sex ratio and should be ideally devoid of any pathology that would render ethically impossible a colon sample resection. This is the reason why we chose the sigmoiditis population as a control. In this population, the resection of the colon was performed when the inflammation was cured, the mucosa was macroscopically normal, and the regimen of these patients was not strikingly or systematically different from that of the patients with colorectal carcinoma. Moreover, both populations (sigmoiditis and colorectal cancer) are perfectly comparable in terms of age and sex ratio. Finally, the population devoid of any known pathology, the organ donors, is much younger and has no detectable adducts. This absence of adducts could be due to age; however, two patients from this control population were older than than 40 years (41 and 48 years), and in colorectal cancer population, three patients were younger than 50 years (43, 44, and 48 years) and had high levels of adducts (46.05, 200.5, and 226.6 adducts/10^9 nucleotides, respectively). Moreover, no correlation was found between the number of adducts and the age in any population (data not shown). Therefore, we think that the high level of DNA adducts in nontumoral mucosa is quite relevant, because it was not found in either of the two control populations.

Our results are, thus, the first to demonstrate, in humans, the presence of numerous adducts in nontumoral colon mucosa of patients with colorectal cancer. Moreover, they are in good agreement with the important role of dietary and environmental carcinogens in colorectal carcinogenesis (1, 5), in terms of exposition and of bioactivation. However, although our results will need to be confirmed in a larger population, they suggest that, at the time of colonoscopy or surgical resection of part of the colon, some colonic cells could be taken, and DNA adduct levels could be determined. This is a simple

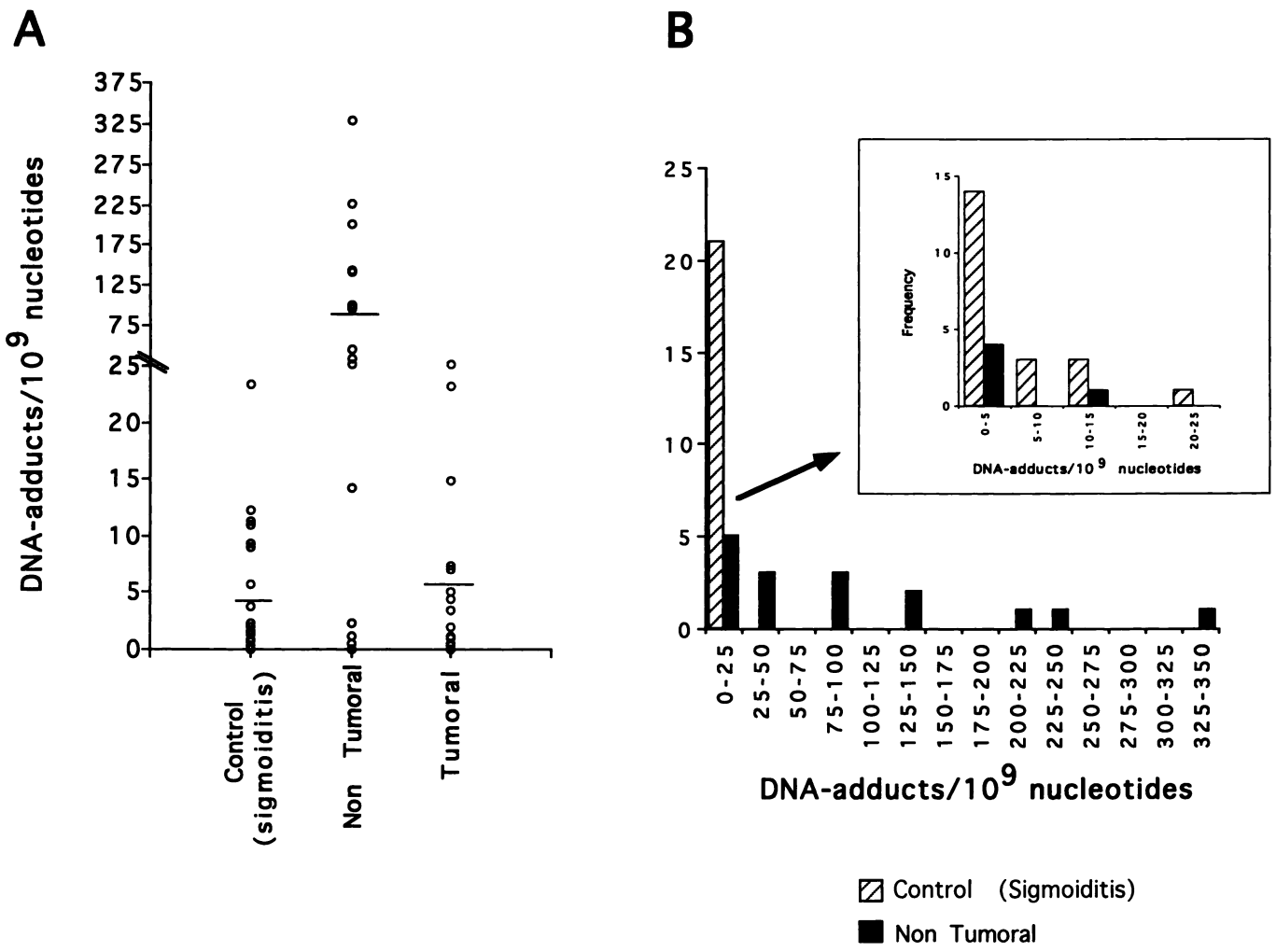

Fig. 2. *A*, DNA adducts in control, nontumoral, and tumoral colorectal tissues; *B*, frequency distribution of DNA adducts from control (sigmoiditis) and nontumoral colorectal tissues.

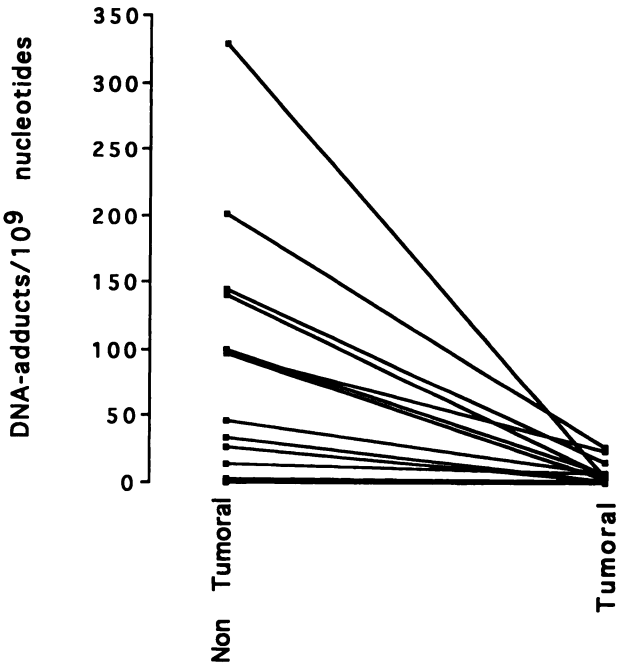

Fig. 3. DNA adducts in human nontumoral and tumoral colorectal tissues from the same patients.

procedure, because even paraffin-embedded tissues, if fixed less than 48 h in formalin, are quite suitable for ^{32}P-postlabeling analyses (30). The detection of high DNA adduct levels (>25 adducts/10^9 nucleotides) could lead to close supervision of the patient by means of regular colonoscopy, with the same time interval as recommended in Lynch syndrome. Indeed, Fig. 2 shows that 69% of the colorectal population have more than 25 adducts/10^9 nucleotides, whereas no patient with cured sigmoiditis has such an adduct level. The detection of DNA adducts 6, 13, and 14, which are observed only in the nontumoral colon, would strengthen the significance of this measurement.

The aim of future investigations will be to identify the adducts and the carcinogens that bind specifically to DNA in epithelial colonic cells and, in particular, those leading to DNA adduct 1. Such a characterization could increase our knowledge of the carcinogens truly implicated in human colorectal carcinogenesis and the process of their elimination.

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