Fecal Neutral Steroids in Normal Conditions and in Patients with Polyps or Cancer of the Large Bowel

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ABSTRACT

There is evidence suggesting that the excretion and conversion of neutral steroids in the human large bowel might be somewhat related to the development of colorectal cancer. Therefore, our objectives were: (a) to characterize the excretion and the major pattern of steroid degradation in normal conditions, both in children and in adults; and (b) to investigate if abnormalities of these parameters are frequent in patients with colorectal cancer or polyps. The study has been carried out in: (a) 38 adult volunteers; (b) 29 children divided into 4 age groups; (c) 22 patients with colorectal cancer; (d) 16 members of 6 families with adenomatosis coli; (e) 15 members of 2 families with a high prevalence of multiple polyps or the large bowel; (f) 12 subjects with colorectal polyps, without familiality. The subjects were kept under metabolic control, fecal samples were collected for at least 3 days and analyzed by thin layer chromatography and gas-liquid chromatography. Total neutral steroid excretion was lower in children than in adult volunteers; in contrast, there was no significant difference between the latter and the other investigated group of patients with cancer or polyps, with values ranging between 230 and 680 mg/day. All the adult volunteers were "high converters" of cholesterol to its intestinal metabolites coprostanol and coprostanone [89 ± 10% (SE) of degradation]. Children less than 1 year old degraded little or no cholesterol [10.4 ± 6% of total neutral sterols], whereas with increasing age the fraction of conversion became more similar to that of adults. In patients with colorectal tumors 2 populations could be defined, one characterized by a large degradation of cholesterol and the other by little or no conversion. Low degradation of cholesterol was found in 3 of 6 families with adenomatosis coli. In conclusion, we did not find any significant differences in total neutral steroid excretion among controls, colorectal cancer patients, or subjects at risk. In adult volunteers the normal pattern of cholesterol degradation is characterized by a large conversion of cholesterol to its intestinal metabolites. In children this process changes with increasing age from an absolute "nonconverting" state (after birth) to the pattern typical of adults. Finally, in a minority of patients with either polyps or cancer of the large bowel and of their first-degree relatives, cholesterol is poorly degraded and represents the most abundant fecal sterol.

INTRODUCTION

Colorectal cancer continues to be a major cause of death in western and developed countries (1). Although the pathogenesis of the disease is largely unknown, epidemiological studies suggest a relationship with dietary factors; in particular, the consumption of animal fat, of meat, and of some alcoholic beverages seems to be somewhat related to the prevalence of the disease (2-5). These observations have been further supported by some animal studies (6,7), but not without controversies (8).

The reasons why certain components of the typical western diet seem to induce an excess of morbidity for colorectal cancer are still poorly understood. Hill et al. (9) showed that European subjects, used to eating diets rich in meat and animal fat, excreted feces containing higher concentrations of both acidic and neutral sterols as compared to people living in African and Asian countries. In the former group bile acids were more largely degraded to secondary products, especially deoxycholic acid. The authors speculated that cancer of the large bowel might be related to high concentrations of fecal derivatives produced by anaerobic bacteria (10-13). Although Reddy and Wynder (14) were led to similar conclusions, other studies failed to confirm these views (15,16). Some of these discrepancies can be explained, at least in theory, by the fact that in most of these studies the investigated subjects were not under metabolic control so that many external factors might have influenced the results.

More recently the studies have been extended to subjects with inherited adenomatosis coli and colon cancer and to their first-degree relatives; Reddy et al. (17) showed that patients with familial polyposis excreted higher levels of undegraded fecal cholesterol than did normal controls. In the same patients differences in the metabolic activity of the microbial flora have been reported. Lipkin et al. (18) studied fecal neutral steroid composition in families with site-specific, inherited colon cancer; they found that the degradation of cholesterol to coprostanol, coprostanone and other secondary metabolites was significantly reduced in both affected individuals and asymptomatic first-degree relatives. The authors suggested that the cholesterol degradation pattern might represent a marker of genetic predisposition to colonic cancer, potentially useful in the surveillance of individuals at risk.

The present study was designed to further clarify the excretion and the major degradation pattern of fecal neutral sterols in both physiological and pathological conditions with subjects maintained under metabolic control. The main purposes were 3-fold: (a) to quantitate the fecal excretion and to evaluate the extent of neutral steroid degradation in a large group of adult subjects; (b) to investigate if there are differences in the pattern of neutral steroid excretion and conversion in children of different age; and (c) to ascertain if abnormalities in the excretion and/or degradation of neutral sterols are more frequent in patients with colorectal cancer, or in individuals at risk of this disease, than in the general population.

MATERIALS AND METHODS

Subjects. The study has been carried out in: group 1, 38 adult subjects (males, 33; females, 5; age range, 20-65 years); group 2, 29 children (males, 15; females, 14; age range, 2 months-13 years); group 3, 22 patients with colorectal cancer (males, 14; females 8; age range, 42-77 years); group 4, 16 members (males, 12; females, 4; age range, 18-65 years), 3 affected and 13 first-degree relatives, of 6 families with adenomatosis coli; group 5, 15 members (males, 9; females, 6; age range, 19-68 years) of 2 families with a high prevalence of multiple polyps and colorectal cancer; group 6, 12 subjects (males, 9; females, 3) with solitary or multiple colorectal polyps, without familiality. Thus, a total of 132 individuals were studied between 1981 and 1984. Rep-
representative pedigrees of families of groups 4 and 5 are shown in Fig. 1. Subjects of groups 1 and 2 were studied during their hospitalization for mild diseases or a control of the general clinical state. Mild arterial hypertension, hypertriglycerideremia, dyspepsia, and Gilbert's syndrome was the most frequent clinical diagnosis in adults; convulsions, mild anemia, and arthritis were the most common clinical conditions encountered in children. Patients with gastrointestinal or hepatobiliary diseases were excluded from the study. At the time of the study the patients were not given any specific drug with the exception, in a few subjects only, of vitamin supplementation, digestive enzymes, and blood (or its derivatives). Particular care was taken in avoiding antibiotics or any other drug potentially capable of interfering with the physiological excretion and degradation of the neutral steroids. Children were divided into 4 groups: group A (N = 9), 2 to 12 months old; group B (N = 7), 1 to 3 years old; group C (N = 6), 4 to 7 years old; and group D (N = 7), 8 to 13 years old.

Patients of group 3 (colorectal cancer) were studied during their hospitalization before surgery. Patients severely affected, markedly constipated, or treated with antibiotics (or any other drug which could interfere with the intestinal metabolism of steroids) were not included in the study. Subjects in groups 4, 5, and 6 were referred to our unit from the endoscopy centers of Modena and Reggio Emilia. Arrangements were made to meet each of the families (or single individuals, as for group 6) at their own homes. This strategy was chosen mainly to put the family members at their ease and to further demonstrate our interest in the study, enabling us to talk to most of the first-degree relatives together. During the interview the clinical records of each individual were examined and a genealogical tree was drawn. When a dominant pattern of inheritance was suspected, endoscopy was recommended to all the first-degree relatives. Then we explained to the family members the purpose of our study and we suggested that they followed the main meals. From the subsequent day all fecal samples were collected for 3 to 6 days in plastic bags and immediately stored at -20°C. In the first 10 adults who were investigated and in 4 patients with colorectal cancer, 2 μCi of β-[14C]-sitosterol were given with each meal to correct for losses of neutral steroids due to rupture of the cyclopentenophenanthenring and consequent incomplete recovery. However, when we simultaneously corrected for fecal flow with Cr2O3 we could ascertain that under the conditions of our study the recovery of β-sitosterol was almost complete. These findings confirm previous observations (22) suggesting that losses due to complete degradation of neutral steroids are uncommon in subjects eating solid diets. Consequently in the rest of the patients radioactive β-sitosterol was not used.

Analytical Procedure. Feces were thawed and each daily sample was analyzed separately. When there was more than one specimen per day they were pooled in a single sample. Total and individual neutral steroids were isolated and quantitated as described previously on repeated occasions by Miettinen et al. (23, 24). Briefly, feces were homogenized with approximately an equal volume of water and 5-10-ml aliquots of the homogenate were taken for saponification. This was carried out in 20-30 ml of 1 N NaOH in 90% ethanol by refluxing for 1 h at boiling temperature. After cooling the neutral steroids were extracted three times in petroleum ether. The combined extracts were dried, redissolved in ethanal diethyl ether (50:50), and applied on Silica Gel G-coated thin layer chromatography plates. These were developed in petroleum ether:diethyl ether (50:50). The areas corresponding to cholesterol, coprostanol, and coprostanone were identified with appropriate standard (after exposure to iodine vapors) and scraped off, and the steroids were extracted from the silica gel by refluxing twice in 150 ml of ethanal diethyl ether (50:50). After evaporation of the solvent, 50 μg of the internal standard (5α-cholestanone) were added to each sample. The hexafluoroisopropyl derivatives were obtained by incubating each sample at 37°C for 30 min in a mixture of hexafluoroisopropanol (100 μl) and trifluoroacetic anhydride (200 μl) (25). The derivatives were redissolved in ethyl acetate and small aliquots (1-3 μl) were injected into a Carlo Erba Gaschromatograph (Fractovap 4200) equipped with 200-cm long spiral columns (2 mm internal diameter) and packed with 3% SP-2401 on Supelcoport (100-200 mesh). The peaks corresponding to the major fecal neutral steroids were identified by appropriate standards and quantitated by comparing the peak area to that of the internal standard. Further details of the analytical procedure have been given in previous studies from our unit (19, 20, 26). The recovery of [14C]-cholesterol added to the initial homogenate throughout the whole procedure was on the order of 75-80%; although not complete this fraction was rather constant in repeated measurements. The results were expressed in mg of each major fecal sterol (cholesterol, coprostanol, coprostanone) per day and as total neutral steroids by pooling the values obtained in each individual day of fecal collection. From the initial homogenate Cr2O3 was measured in 1-5-ml aliquots according to the method of Bolin et al. (27).

Statistical Analysis. The results are expressed as the mean ± SE. The statistical significance of differences between means was evaluated with Student's t test for unpaired analysis.

RESULTS

The excretion and conversion of fecal neutral steroids in adults without colorectal neoplasms (group 1) and children of different ages are shown in Table 1. The excretion of neutral steroids was significantly lower in children of groups A and B (less than

Fig. 1. Representative pedigrees of a family with adenomatosis coli (A) and a high prevalence of multiple polyps and colorectal cancer (B).
Table 1  Daily excretion of fecal neutral sterols in adult volunteers without colorectal neoplasms and in children of different ages (mg/day ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol</th>
<th>Coprostanol</th>
<th>Coprostanone</th>
<th>Total sterols</th>
<th>Cholesterol (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A (2–12 mo, N = 9)</td>
<td>123 ± 41*</td>
<td>12 ± 8*</td>
<td>2 ± 2</td>
<td>137 ± 41*</td>
<td>90</td>
</tr>
<tr>
<td>Group B (1–3 yr, N = 7)</td>
<td>107 ± 31</td>
<td>132 ± 85</td>
<td>10 ± 9</td>
<td>249 ± 92</td>
<td>43</td>
</tr>
<tr>
<td>Group C (4–7 yr, N = 6)</td>
<td>99 ± 45</td>
<td>215 ± 72</td>
<td>4 ± 2</td>
<td>318 ± 60</td>
<td>31</td>
</tr>
<tr>
<td>Group D (8–13 yr, N = 7)</td>
<td>110 ± 73</td>
<td>234 ± 39</td>
<td>12 ± 5</td>
<td>356 ± 89</td>
<td>30</td>
</tr>
<tr>
<td>Adults (20–65 yr, N = 38)</td>
<td>49 ± 7</td>
<td>346 ± 45</td>
<td>29 ± 6</td>
<td>424 ± 45</td>
<td>11</td>
</tr>
</tbody>
</table>

* P < 0.01 at least versus the adult group.

Fig. 2. Intestinal degradation of fecal neutral sterols in the control group. Individual values are given for each of the three major sterols as percentage of the total.

1 and 3 years old, respectively) as compared to adults. Values observed in groups C and D were closer to those of the adult population. When individual sterols were considered, cholesterol was the most abundant fecal sterol in children less than 1 year old; on the average it represented 90% of total neutral sterols with a range of 60 to 100%; thus, all the subjects of this group were low or nonconverters of cholesterol to its metabolites and the sum of the fractions of the 2 degradation products was only 10% of the total. In the other 3 groups of children cholesterol progressively represented, with increasing age, a lower fraction of total neutral sterols. There were 3 “low converters” of cholesterol (i.e., fractional sum of coprostanol plus coprostanone, <40% of total neutral sterols) in group B, 2 in group C, and 2 in group D. In the adults the excretion of fecal cholesterol was even less (49 ± 7 mg/day) and its fraction was reduced to 11% of total neutral sterols; we did not find any low converter of cholesterol in this group. As shown in Table 1, the pattern of excretion of coprostanol plus coprostanone was opposite of that of cholesterol; the 2 major intestinal catabolites were almost absent in the children of group A but became progressively more represented with increasing age. In the adults the normal pattern of degradation is illustrated for each patient in Fig. 2; in none of the subjects did cholesterol reach 40% of the total, whereas the sum of coprostanol plus coprostanone was always >60% of total neutral sterols. Thus, under the conditions of our study, the normal pattern of neutral steroid degradation was the so-called “high converter” state (28). Since we studied each patient for several days and analyzed each daily fecal sample separately, we could evaluate the fluctuation of the degradation pattern throughout the study; actually this was negligible and a given conversion pattern remained markedly stable during the study, with changes of <5% from one day to another. In addition, we had the opportunity to study 3 patient (adults) during a second hospitalization. Again, the degradation pattern did not differ from the values observed previously. These findings fully confirm the stability of the conversion state, a concept which was already suggested by Wilkins and Hackman (28) in their original paper. The excretion of plant sterols was very low, presumably because the standard hospital diet did not provide large amounts of vegetables; in the adults, the daily fecal excretion of β-sitosterol was 6.9 ± 2.3 mg/day and that of campesterol was 2.2 ± 1.2. In children these 2 (and other) plant sterols were virtually absent or present in trace amounts. Fig. 3 summarizes the degradation pattern of cholesterol in the various age groups. The values for children younger than 1 year and those for adults represent the 2 extremes, one characterized by a virtual lack of conversion of cholesterol and the other by an almost complete degradation to coprostanol and coprostanone.

The excretion of total neutral sterols in patients with colorectal cancer or polyps is shown in Table 2. A great scattering of results was observed within each group with values ranging from 200 to nearly 700 mg/day. There was a large overlapping of values between the groups and no difference (between controls and each patient group) reached statistical significance.

Adult subjects without colorectal neoplasms (Table 1; Fig. 2) were high converters of cholesterol into its intestinal metabolites coprostanol, coprostanol, and coprostanone (mean degra-
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Fig. 4. Intestinal conversion of fecal neutral steroids in the four groups of patients with colorectal neoplasms and in adult volunteers. The results (mean ± SE (bars)) are given as percentage of total neutral steroids present in feces. Ⅲ, cholesterol; □, cholesterol plus coprostanone; NS, not significant.

Fig. 5. Degradation of fecal neutral steroids in patients with colorectal cancer. A. A sampling percentage of degradation (i.e., the fraction of cholesterol which was degraded to coprostanol and coprostanone).

Fig. 6. Degradation of fecal neutral steroids in families with adenomatosis coli (see Figs. 1 and 3).

Figs. 5–8 show the degradation pattern in more detail. In each investigated group two distinct patterns could be identified, one showing a conversion similar to that of controls (i.e., >60% degradation of cholesterol to coprostanol and coprostanone, high converter), the other (low converter) being characterized by little conversion of cholesterol (<40%). Among the patients with colorectal cancer (Fig. 5) there were 15 high converters, 5 low, and 2 “intermediate,” with degradation between 40 and 60%. Among subjects of group 4 (i.e., members of families with adenomatosis coli) there were 10 high converters and 6 low converters (Fig. 6), found in only 3 of 6 families. Of the 6 low converters, 3 had a negative endoscopy, 1 had polyposis, and the remaining 2 refused any investigation. Of the 10 high converters, 2 had polyposis, 6 had a negative colonoscopy, and 2 refused the endoscopy. When restudied on a second occasion 2 low converters showed an unchanged pattern of conversion. In group 5 (Fig. 7) the majority (13 of 15) of the subjects were high converters, including those individuals with recurrent multiple polyps and with colorectal cancer; the low converters were 2 young subjects (20 and 22 years old), asymptomatic and with a negative endoscopy. Surprisingly, 4 of 12 subjects with polyps without familiality were low converters of cholesterol; the existence of two patterns of degradation is shown in Fig. 8. As already shown in subjects of group 1, the excretion of plant steroids or other degradation products was negligible and no consistent difference was observed among the investigated groups.

DISCUSSION

The most relevant findings of the present study can be summarized as follows. (a) In adults without colorectal neoplasms
kept under metabolic control the intestinal metabolism of neutral sterols is characterized by a large conversion of cholesterol to coprostanol and coprostanone. This has been observed in 38 consecutive subjects and in each of the collected and analyzed fecal samples. Furthermore, the conversion pattern was stable when studied on a second occasion many months after the previous determination. (b) In children less than 1 year old nondegradation of cholesterol in feces is commonly observed; when the children grow older some of them begin to metabolize the luminal cholesterol to the same extent as adults and eventually, as can be inferred from Fig. 3, at adolescence and, presumably, in the years immediately after, the degradation pattern of cholesterol becomes virtually that of the adults. (c) Patients with colorectal cancer or at risk for these malignancies (polyps, adenomatosis coli), kept under metabolic control, do not excrete higher amounts of neutral sterols in feces when compared to a control group presumably representative of the general population. (d) In a minority of patients with either cancer or polyps of the large bowel cholesterol is poorly degraded and represents the most abundant fecal sterol; this nondegradation of cholesterol, although more frequent in adenomatosis coli families, was also observed in the other groups of patients in whom large bowel neoplasms do not seem genetically determined.

Our data are in agreement with those of Gustaffson and Wernez (29) who showed little or no conversion of cholesterol in infants less than 1 year old but are at variance with the findings of Wilkins and Hackman who found two patterns of neutral sterol degradation in normal adults (high converters and low converters, as already mentioned) (28). The reasons for these differences are unclear; it is likely that the existence of only one pattern of degradation in our adults reflects the metabolic control achieved in these subjects who ate a standard diet and were kept in the hospital for the entire period of the study. On the other hand, the two patterns of conversion reported by the above mentioned authors might be due to the effects of other factors, such as the consumption of alcoholic beverages, coffee, smoking, physical activity, or occupation, which were not controlled and the effect of which on the “microbial ecology of the intestine” has not yet been studied (18).

There is little doubt that the composition of the intestinal bacterial flora plays a major role in influencing the rate and extent of neutral sterol degradation. Set in a different way, therefore, our data indicate that the microbial flora of infants, or at least its metabolic activity, is somewhat different from that of older people; as the children mature, however, it is likely that their intestinal bacterial composition becomes more similar to that of the adult population.

Previous studies suggested that the fecal concentration of acidic and neutral sterols was higher in patients with or at risk of colorectal cancer than in the general population (13, 14). Even assuming that neutral sterols might be somehow related to the risk or the development of colorectal cancer, the measure of their concentration in casual stool samples may appear as an inaccurate indication of the cocarcinogenic effect. The intestinal content, in fact, may be more or less concentrated in different segments and at different times; therefore we do not know how fecal concentration may compare with that of the various large bowel tracts. This aspect seems even more relevant when patients are not under metabolic control as is in the above mentioned studies. As has been pointed out in a recent editorial (30) most studies on this topic have been done on fecal samples only because feces are easily obtainable, but there is still no evidence that fecal flora or fecal metabolites are representative of those of the cecum and right colon. To overcome these problems, at least in part, we gave our subjects a standard diet, with few but well defined limitations, and studied most of them during their hospitalization, so that many variables (alcohol consumption, coffee drinking, changes of body weight) could easily be controlled. In addition we expressed our results as mg of total neutral sterols excreted per day; this measurement reflects the overall intestinal flow of neutral sterols, which cannot be inferred by the sole determination of their fecal concentration. Under the conditions of our study we failed to show any consistent increase of fecal neutral sterol excretion in patients with colorectal cancer and in patients with various types of polyps, who probably represent different classes of individuals at risk of intestinal malignancies.

Our studies confirm the findings of Reddy et al. (17) and Lipkin et al. (18) who showed that patients with adenomatosis coli and their first-degree relatives tend to excrete high amounts of undegraded cholesterol and less coprostanol and coprostanone when compared to controls. This abnormality, however, was observed in only 3 of the 6 investigated families; in addition, only 1 of the low converters detected was undoubtedly affected, while the others were asymptomatic relatives. Our data also suggest that the low conversion pattern, uncommon in adult volunteers, may be encountered rather more frequently in other groups of subjects at risk of colorectal cancer as well as in patients affected by this malignancy. The factors leading to reduced degradation of cholesterol in some individuals in the 4 investigated groups are not entirely clear. Undoubtedly the intestinal bacterial flora plays a major role in determining the extent of sterol degradation, but the possibility that intestinal cells themselves take part in luminal cholesterol metabolism cannot be excluded a priori (18). Our study confirms previous findings on the low degradation of cholesterol in families at genetic risk of colorectal cancer (adenomatosis coli, site-specific inherited cancer) (17, 18) but these observations are extended to clinical conditions without any apparent genetic factors, as in the case of our patients in group 3 (colorectal cancer) and in group 6 (solitary or multiple polyps) where no familial aggregation could be detected.

Finally, one might wonder if the study of the degradation pattern of cholesterol in stool specimens may be of help in identifying persons at risk of colorectal cancer and in the long term surveillance of these subjects. Lipkin et al. (31) have recently defined a “risk profile” for colorectal cancer based on clinical and biological parameters; among the latter, together with the incorporation of [3H]thymidine in crypt cells of colorectal mucosa, carcinoembryonic antigen in colonic lavage, mixed leukocyte response, and cutaneous fibroblast culture, the authors underlined the role of the “fecal parameters” (i.e., the percentage of undegraded cholesterol and the proportion of cholic acid metabolized to deoxycholic acid). Our findings give further support to this contention and suggest that the low conversion of cholesterol in feces might be a useful index, in a minority of subjects and together with other parameters, of an increased susceptibility to malignancies of the large bowel. Additional information, however, is still lacking and should be researched with care; for instance, we still know relatively little about the physiological factors which might influence the intestinal degradation of cholesterol; finally, longitudinal studies are needed to ascertain if the number of colorectal cancers occurring in subjects with a low degradation of cholesterol is higher than that of the general population.
REFERENCES

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