Are bile acids carcinogenic?

A critique

by Dr. M. B. KATAN*

Summary. *Some of the evidence for the putative carcinogenicity of bile acids is reviewed. It is argued that, contrary to what has been suggested*
1. bile acids bear no structural similarity to polycyclic aromatic hydrocarbons;
2. human intestinal bacteria have not been shown to convert bile acids into carcinogens;
3. the carcinogenicity of bile acids in animal experiments is unproven.

There is some tentative evidence that bile acids have a co-carcinogenic, or tumour promoting potential. Such a potential, however, can be expressed only in the presence of a true, primary carcinogen. As yet there is no convincing proof for the presence of such carcinogens in the human intestine. It is concluded that knowledge about the role of diet and bile acids in the causation of large bowel cancer is not yet at a stage where specific dietary recommendations to the public at large are justified.

Epidemiological studies on the distribution of cancer of the large bowel have suggested large differences in the prevalence of colon cancer between different parts of the world. These differences appeared to be independent of climate and race (19), and it therefore seems logical to suppose that environmental factors such as nutrition play a part. Several aspects of the Western diet have been implicated in colon carcinogenesis; among these are a high intake of saturated fat (19) and animal protein (8), and a low intake of dietary fibre (1,17).

In most hypotheses on the mechanism relating nutrition with the induction of colon tumours, bile acids play a central role. Originally this was probably inspired by the discovery, now 48 years ago, that deoxycholic acid and cholic acid can be converted *in vitro* (18) into methylcholanthrene, a very potent carcinogen. This led to the suggestion that a similar conversion might take place in the body and be responsible for various types of cancer. Animal experiments, some of which will be discussed below, seemed to confirm the carcinogenic action of bile acids. As for the role of diet, a high fat consumption is thought to increase the amount of bile acids excreted (4), and a low fibre diet decreases the volume of the faeces and thus increases the concentration of bile acids present in them. Low-fibre diets also prolong the time of residence of the faecal bolus within the intestines by increasing transit time. This would allow intestinal bacteria more time to produce harmful substances, and it would lengthen the time of contact of these substances with the intestinal mucosa.

Additional evidence for the bile acid-cancer hypothesis is supplied by studies on faecal steroids and faecal bacteria in people from different parts of the world, and in people suffering from colon cancer. It was found that bile acid concentrations are higher in faeces from cancer patients when compared with controls (12), and higher in subjects from affluent countries like England when compared with people from tropical countries (11). The latter also have lower amounts in their faeces of certain anaerobic bacteria that are thought to be responsible for the conversion of bile acids into noxious substances.

The hypothesis as briefly outlined above is attractive both because it gives an explanation for the observed differences in colon tumour incidence between affluent and tropical countries, and because it suggests means to prevent this disease, which is at present in the USA and Western Europe the most frequent malignant neoplasm after lung cancer. There are, however, a number of weaknesses in the evidence for this hypothesis which, in the opinion of this author, have received too little attention. It is the aim of this paper to review some of these weak points.

1. Are bile acid molecules structurally similar to carcinogens?

It has been repeatedly stated that certain bile acids are sterically similar to carcinogenic polycyclic aromatic hydrocarbons (11, 15, 16). A closer look at the structure of these compounds shows that this statement needs some qualification. Structural formulas generally give a two-dimensional representation of molecules. For methylcholanthrene and other polycyclic aromatic hydrocarbons this is no problem because its carbon skeleton is flat, and therefore the plane projection (structure Ia in fig. 1) is quite similar to the spatial view (structure Ib). However, for saturated (aliphatic) polycyclic carbon compounds, such as bile acids and steroids, the usual plane projection (structure IIa in fig. 1) gives a false impression. The spatial conformational drawing of deoxycholic acid (IIb) shows that bile acids are in fact not flat like polycyclic aromatic hydrocarbons, but instead consist of pleated cyclohexane rings attached to each other at an angle. The difference in 'flatness' between bile acids and polycyclic aromatic hydrocarbons is relevant because many authors now believe (9) that the mutagenic and carcinogenic properties of polycyclic aromatic hydrocarbons are caused by their interaction with DNA, and insertion of the flat aromatic molecule between the flat aromatic bases of the DNA chain is a mechanism proposed for this (9). For bile acids such an insertion seems unlikely, both because of the

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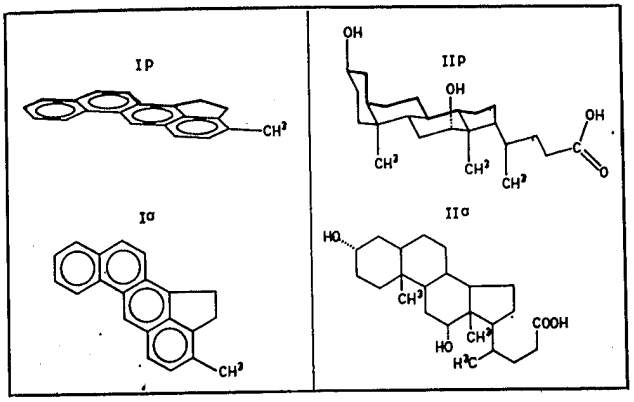

Fig. 1. Comparison of the structure of the carcinogen methylcholanthrene (Ia and Ib) and the bile acid deoxycholic acid.
Ia and IIa are plane projections, and Ib and IIb are three-dimensional, spatial views. Note the difference between the true conformational structure IIb of the bile acid and the commonly used flat projection, IIa.

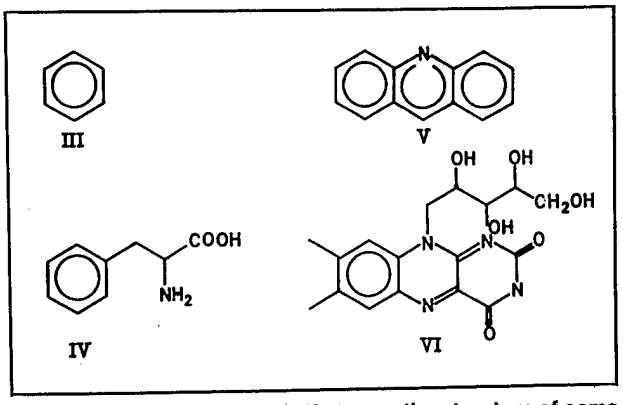

Fig. 2. The apparent similarity between the structure of some beneficial aromatic compounds and of some aromatic toxic or mutagenic substances.
III, benzene; IV phenylalanine, an essential amino acid; V, acridine, a mutagen; VI, riboflavine (vitamin B_2).

bulk of the molecule and because of the absence of aromatic rings.

The ultimate carcinogenic forms of most, if not all, chemical carcinogens are strong electron-deficient or electrophilic reactants (14), with an affinity for nucleophilic (electron-rich) centres in cellular macro-molecules. However, neither primary bile acids nor their usual bacterial metabolites could be considered electrophilic reactants.

Thus, the similarity between certain bile acids and carcinogenic polycyclic aromatic hydrocarbons claimed by several authors (11, 15, 16) is only superficial and not real. The similarity is certainly insufficient for ascribing to bile acids the carcinogenic properties of e. g. methylcholanthrene.

2. Can intestinal bacteria convert bile acids into carcinogens?

Ever since WIELAND and DANE effected the chemical conversion of deoxycholate into methylcholanthrene there has been a search for the formation of this carcinogen *in vivo*. Until now the results have been negative. HILL and coworkers (6) showed that intestinal bacteria can convert 4-androsten-3, 17-dione into 17-methoxy-1, 3,5 (10) -estratriene-3-ol by aromatization of the A-ring. The formation of steroid compounds with two aromatic rings has also been described (7). Thus, certain artificial steroids can be converted *in vitro* by bacteria into the corresponding compounds with one or two aromatic rings. Neither the steroid precursors nor the aromatic products were reported to be present in the human intestine, and no carcinogenicity tests of the products were mentioned. In summary, there is no evidence that bile acids are converted into carcinogens in the human gut.

The fact that the compounds produced thus from steroids were aromatic has by itself been regarded sometimes as support for a role of bile acids in colon carcinogenesis. In this context it should be noted, however, that aromatic compounds as such are not necessarily harmful. Indeed, a large number of aromatic substances are naturally present in living organisms, and the human body absorbs many of these from food in the form of vitamins and essential amino acids. Two examples are shown in fig. 2.

The essential amino acid phenylalanine (structure IV) is a derivative of benzene (structure III), which by itself is quite toxic. Vitamin B_2 (structure VI) is a tricyclic aromatic compound with a ring system that has some resemblance to acridine (structure V), a well-known mutagenic compound. Many more nutrient compounds could be cited which are aromatic and beneficial at the same time. It is only a very restricted group of aromatic compounds that causes tumours, and even within this group small changes in structure can cause large differences in carcinogenic potential. Hence, the bacterial conversion of steroids into compounds with one or two aromatic rings (6, 7) by itself means very little.

3. Are bile acids carcinogenic in animals?

Some of the studies investigating the induction of tumours by bile acids in experimental animals are reviewed below. The list is certainly not exhaustive, but the papers discussed are all quoted frequently, and for that reason alone merit closer attention.

3.1 COOK et al. (2) described an experiment in which deoxycholic acid dissolved in sesame oil was injected into ten mice. Of the five mice that survived for more than six months, three developed spindle-celled tumours at the site of injection. These were regarded as malignant on histological grounds, but did not grow when transplanted. The mice had received 15 injections containing in all 70 mg of deoxycholic acid in 300 days. In a later experiment ten mice of a different strain were injected in a similar manner and one of these was reported to have developed a tumour 155 days after receiving a total of 28 mg of deoxycholic acid. Unfortunately, the authors did not employ control groups of any kind to check for e.g. the action of the solvent used or the spontaneous emergence of tumours. Also the number of animals employed was small, and no statistical analyses whatsoever were performed to check whether the number of tumours seen might be due to chance. Finally, the route of entry appears unsuitable for testing a substance that is supposed to act by direct contact with the inside of the intestinal wall.

3.2. COOMBS et al. (3) synthesized a number of aromatic hydrocarbons with the same carbon ring structure as bile acids, and applied them to the skin of albino mice. It was

found that out of the seven closely related compounds that were tested, one, 11-methyl-15, 16-dihydro-17-oxo*cyclo*-penta(a)phenanthrene showed marked carcinogenicity. This substance lacks the angular methyl groups at C-10 and C-13 that are characteristic for steroids, including cholesterol and bile acids. Thus, it cannot be regarded as a proper steroid derivative, and its formation from bile acids or sterols *in vivo* would require drastic molecular rearrangements.

3.3. DRUCKREY et al. (5) investigated the effect of deoxycholic acid and of dehydronorcholene, which is an intermediate in the chemical synthesis of methylcholanthrene. Both were injected subcutaneously into rats, either as such or after pre-incubation of the test compounds with cultures of the intestinal bacterium *Escheria coli.* Tumours arose in animals treated with pre-incubated dehydronorcholene; they were found predominantly at the site of injection. A tumour at the site of injection also arose in an animal in a control group treated with an extract of *Escheria coli* alone. No tumours were found in animals injected with deoxycholic acid, either with or without bacterial incubation.

3.4. LACASSAGNE et al. (13) injected the steroid compounds 7-dehydrocholesterol acetate and 3β-acetoxy-bisnor△5-cholenic acid subcutaneously in mice. With the first compound, three out of sixty animals developed sarcomas at the site of injection, and with the second compound two out of sixty did so. Control experiments with solvent alone and with certain other steroids were uniformally negative. It is thus safe to assume that the compounds tested are at least weakly sarcomagenic upon subcutaneous injection. However, the presence of these compounds in the gut *in vivo* was not documented.

It is this author's opinion that none of the studies mentioned above ought to be cited as evidence that human bile acids are carcinogenic in animals. Several more recent studies have also failed to confirm a direct tumour-induction by bile acids in animals (16), and some investigators are now concentrating instead on the interaction of bile acids with known carcinogens. Indeed it was shown that intrarectal instillation of either primary or secondary bile salts will increase the number of tumours caused in rats by synthetic carcinogens like N-methyl-N'-nitro-N-nitroso-guanidine (16). Such experiments point towards a co-carcinogenic, or tumour promoting activity for bile acids. They would thus need the presence of a substance of known carcinogenic potential to become noxious.
Research about the nature of this hypothetical primary carcinogen in man is in progress (10), but there is no agreement about its identity or even its existence. Hopefully future research will shed more light on this question.

Conclusion

The suggestion that bile acids have carcinogenic potential or can be transformed *in vivo* into carcinogens has received widespread attention. As urged in this paper, the theoretical and experimental basis for this aspect of the nutrition-colon cancer hypothesis is rather tenuous.
There is some evidence for a co-carcinogenic, or tumour promoting effect of bile acids. However, such an effect is only expressed in the presence of true, primary carcinogens, which still remain to be convincingly identified.
Colon cancer is one of the most frequent malignant diseases in western populations, and an environmental origin such as nutritional patterns appears quite plausible. In the opinion of the present author, however, hypotheses linking nutrition to colon cancer via bile acids or their transformation products should be regarded primarily as a source of guidance for new research. A role for dietary factors in colonic carcinogenesis remains to be established, and a mechanism employing bile acids or their transformation products is even more speculative. It is premature at present to suggest to the public at large that a lower intake of animal proteins and fat or a higher intake of dietary fibre will protect against large bowel cancer. If such statements had to be withdrawn ten or twenty years from now this would do great harm to the credibility of nutritional advice in general.

Samenvatting

Galzuren, voeding en kanker van de dikke darm: enige punten van kritiek

Er wordt wel gesteld dat een verkeerde voeding mede oorzaak zou kunnen zijn van het ontstaan van colon kanker. Hierbij zou de concentratie en de samenstelling van galzuren in de dikke darm een belangrijke rol spelen. In dit artikel wordt op een aantal onderdelen van deze hypothese kritiek geleverd. Er wordt betoogd:

1. galzuren hebben geen structurele overeenkomst met polycyclische aromatische koolwaterstoffen.
2. het is volledig onbewezen dat de ingewandsbacteriën van de mens galzuren kunnen omzetten in kankerverwekkende stoffen.
3. het kankerverwekkend vermogen van galzuren in proefdieren is twijfelachtig.

Wel zijn er enige aanwijzingen dat galzuren co-carcinogene, tumorbevorderende effecten kunnen hebben. Een dergelijke activiteit komt echter alleen tot uitdrukking in aanwezigheid van een werkelijk kankerverwekkende stof. De aanwezigheid van dergelijke primaire kankerverwekkende stoffen in de dikke darm van de mens is tot nu toe niet overtuigend aangetoond.
Geconcludeerd wordt dat de kennis van de rol van voeding en galzuren bij het ontstaan van dikke-darm kanker niet in een zodanig stadium is dat specifieke voedingsadviezen aan het publiek gerechtvaardigd zijn. Het zou de geloofwaardigheid van voedingsadviezen ernstig schaden als dergelijke adviezen tien of twintig jaar later weer teruggenomen zouden moeten worden.

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Voedingsgewoonten van jonge Turkse en Marokkaanse kinderen in Nederland*

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Inleiding

Aan de Vakgroep Humane Voeding van de Landbouwhogeschool te Wageningen is door een aantal studenten*** onderzoek verricht naar de voedingsgewoonten van Turkse en Marokkaanse migranten in Nederland. Dit onderzoek vond plaats in samenwerking met het Voorlichtingsbureau voor de Voeding en het Buro Voorlichting Gezondheidszorg Buitenlanders. Deze instellingen zijn met name geïnteresseerd in de voedingsgegevens van peuters en kleuters, omdat zij op basis van deze informatie, indien nodig, voorlichtingsmateriaal willen samenstellen ten behoeve van deze groep.

De doelstellingen van het onderzoek waren als volgt:
- inzicht krijgen in de veranderingen die zich onder invloed van migratie voordoen in de voeding en de voedingsgewoonten van migranten;
- een beeld krijgen van de eventuele problemen met betrekking tot het voedsel en de voeding die door migranten in Nederland worden ervaren of die zich, vanuit voedingskundig oogpunt bezien, voordoen.
- het verschaffen van informatie over voedsel en voeding van migranten, die als uitgangspunt kan dienen voor voorlichtingsactiviteiten van het Voorlichtingsbureau voor de Voeding en het Buro Voorlichting Gezondheidszorg Buitenlanders.

Het onderzoeksteam was van mening dat voor het krijgen van een goed inzicht in de voedingsproblematiek ook kennis nodig is van andere aspecten van het leven van de migranten aangezien de voedingsgewoonten ingebed liggen in een breder kader. Een aantal van die aspecten waaraan aandacht besteed is, waren:
- de leefomstandigheden in Turkije en Marokko;
- de voeding en voedingsgewoonten in Turkije en Marokko;
- de oorzaken van de migratie;
- de problemen van de migranten in Nederland met betrekking tot zaken als huisvesting, arbeidssituatie, communicatie, onzekerheid omtrent de toekomst e. d.

Voordat er met het veldonderzoek begonnen werd, zijn deze onderwerpen door middel van literatuurstudie uitgediept. Naar voren kwam dat de migranten in Nederland de voeding over het algemeen niet als een groot probleem ervaren; zeker niet in verhouding tot andere problemen waarmee ze in Nederland geconfronteerd worden.

Het aantal Turkse en Marokkaanse kinderen in Nederland is de afgelopen jaar sterk toegenomen en zal de komende jaren zeer waarschijnlijk nog aanzienlijk toenemen. Na de wervingsstop van economisch actieven in 1973 vindt legale migratie vooral plaats in het kader van gezinshereniging. De Turkse en de Marokkaanse kinderen in het onderzoek

* Voordracht, gehouden op het symposium 'De voeding van migranten', georganiseerd door de Ned. Ver. voor Voedingsleer en Levensmiddelentechnologie en de Ned. Ver. van Diëtisten. Utrecht, 30 oktober 1980.

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*** Het onderzoek van de Vakgroep Humane Voeding werd uitgevoerd door: G. Buijs, H. van Doorn, S. Essers, P. Glasbergen, J. Groeneweg, M. Kop, M. Schuttelaar, J. Verbeek en C. de Winter. Het onderzoek werd begeleid door: Drs. A. P. den Hartog.