5β-Reduction of [^{14}C]Cholesterol by Human Feces in vitro: Nonspecific Inhibition by Sugars

Coprostanol is the major microbial reduction product of cholesterol excreted in the feces of most animals[1–3] except pigeons[4,5]. Studies by DAM[6] and AMMUNDSEN[7] showed that human beings and other species on milk diets excrete cholesterol exclusively. Feeding lactose leads to a considerably suppressed formation of coprostanol[8,9] with consequent increase in liver concentration[8]. It is not known whether milk or lactose can suppress the conversion of [4-^{14}C]cholesterol into coprostanol in in vitro fecal homogenates. Neither is it known whether other monosaccharides and disaccharides also can inhibit this conversion. We have examined the effect of numerous sugars on the conversion of [^{14}C]cholesterol into [^{14}C]coprostanol by human fecal homogenates in vitro.

Table I. Effect of lactose, galactose and milk on the 5β-reduction of [4-^{14}C]cholesterol by fecal homogenates in vitro[a]

Addition	Decrease in 5β-reduction (%)[b]
Lactose (μmol)	
0.28	40.0
1.40	33.2
2.80	38.5
14.00	37.3
28.00	44.2
70.00	68.7
140.00	66.4
Galactose (μmol)	
0.28	36.9
0.56	24.0
2.80	32.1
5.60	32.5
28.00	43.2
56.00	53.4
140.00	70.8
Milk (ml)	
1	57.4
2	59.2

[a] Fecal homogenates from human subjects were incubated with [4-^{14}C]-cholesterol for 7 days. Various sugars were added in aqueous solution. The formation of 5β-reduction products in different subjects varied from 5.4 to 56.6%. [b] Percentage formation of 5β-reduction products in control tubes is taken as 100.

Table II. Comparative effects of various sugars on the 5β-reduction of [^{14}C]cholesterol in vitro[a]

Sugar added	Decrease in 5β-reduction per mg sugar[b] (%)
Lactose	80.0
Galactose	70.3
Fructose	69.2
Glucose	68.1
Sucrose	82.2

[a] Fecal homogenates from subject were incubated with [^{14}C]cholesterol for 7 days in absence or presence of various sugars. Conversion of control tubes is about 18.5% of cholesterol added. [b] Percentage formation of 5β-reduction products in control tubes is taken as 100.

Method. Fresh stool samples obtained from human subjects were diluted with physiologic saline solution and homogenized. [4-^{14}C]Cholesterol in 25 μl was added to the homogenates and the mixture was incubated anaerobically at 37 °C for 7 days[10,11]. Control tubes contained ethanol or no stool. After incubation the steroids were extracted as described previously[4,5]. The extracts were spotted on thin-layer plates coated with silica gel G along with standard cholesterol, coprostanol, and coprostanone. The plates were developed in a solvent system consisting of heptane-ethyl ether 45:55 (vol/vol) as described by MIETTINEN et al.[12]. The bands corresponding to the standards were located by spraying the plates with 2,7-dichlorofluorescein and scraped off into vials and eluted with chloroform. Aliquots of the eluates were taken for counting. The mass identity of the steroids was confirmed by their behavior on gas-liquid chromatography as described previously[2,3].

Results and discussion. Table I shows the results obtained on adding varying concentrations of lactose and galactose on the 5β-reduction of [4-^{14}C]cholesterol. As can be seen at a level of 0.28 μmol, lactose showed nearly 40% inhibition of the conversion. Further addition up to 70 μmol did not increase the degree of inhibition. When 140 μmol was added a slight increase in the degree of inhibition was noted. A similar effect is also seen with galactose. With both the sugars the degree of inhibition did not parallel the increase in concentration, which suggests that this change is a gradual adaptation to the sugar effect by the microorganism involved in the conversion. Also, the degree of inhibition varied with stool samples obtained from different subjects. If galactose moiety of the lactose was responsible for the inhibition, on a molar basis, galactose should have been twice as active as lactose. However, this is not the case. This finding indicates that the phenomenon of inhibition is general to sugars.

Inhibition by lactose and galactose of the 5β-reduction of cholesterol compared well to that obtained after the addition of milk (Table I) (the concentration of lactose in cow's milk is about 40 to 50 g/l). Further experiments were done to see whether other sugars also can inhibit the 5β-reduction of [4-^{14}C]cholesterol (Table II). As can be seen from Table II all the sugars showed more or less similar effects on a weight basis. This suggests that the inhibition of coprostanol formation is a general effect of all the sugars. The inhibition of coprostanol formation by lactose has also been noted by EYSSEN,

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DEPAUW and PARMENTIER of the Rega Institute, Louvain, Belgium (personal communication). These authors found that lactose inhibits the formation of coprostanol by *Eubacterium* ATCC-21408 (a nova species isolated from rat fecal contents, which has Δ^5-steroid reducing capacity[13]) when it is present in mixed cultures with *Clostridium* (Cl-8), *Escherichia coli* or *Streptococcus faecalis*.

The exact mechanism of sugar inhibition of 5β-reduction is not known. It could be an effect on the specific bacterial flora because of a decrease in the pH of the incubation medium due to the fermentation of the sugar by the bacteria. It also could be a 'catabolite repression effect' of sugars long known in the microbial growth and metabolism[14]. Studies are in progress to elucidate the nature of inhibition by sugars of coprostanol formation. This inhibition could have physiologic significance since 5β-stanol is a major nonabsorbable product of cholesterol excreted in the feces[15].

Zusammenfassung. Nachweis, dass die 5β-Reduktion von [^{14}C]Cholesterol homogenisierter Fäces durch Milch, Laktose und Galaktose gehemmt wurde, wobei die Hemmung unspezifisch war und auch mit anderen Zuckern erzielt werden konnte.

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Biosynthèse des glycoprotéines dans les cellules infectées par un Arbovirus III. Influence des conditions de la multiplication virale et de la concanavaline A

Des travaux antérieurs[1,2,3] ont montré qu'au cours de la multiplication d'un Arbovirus (virus Sindbis) dans les fibroblastes d'embryon de poulet, les activités de biosynthèse glycoprotéinique étaient considérablement augmentées. Le phénomène se manifeste essentiellement au niveau des structures microsomiques. Il est spécialement net dans le cas des glucosaminyl-transférases[1] et des mannosyl-transférases[2,3]. Pour ce dernier système enzymatique, on a montré, en milieu acellulaire de biosynthèse in vitro, les différences significatives entre la mannosyl-transférase des cellules saines et son homologue des cellules infectées, pour ce qui concerne la cinétique, l'influence du pH et de la température, les constantes de Michaelis (substrat GDP-mannose), les effets des cations divalents, des nucléotides et nucléosides-di- et triphosphates, des antibiotiques et des détergents modifiant les structures membranaires (Triton X-100).

Il a paru intéressant de préciser l'influence de plusieurs paramètres de culture cellulaire ou de fonctionnement enzymatique sur cette hyperactivité de glycosylation apparaissant dans les cellules infectées par l'Arbovirus.

Les cellules fibroblastiques d'embryons de poulet âgés de 10 jours sont cultivées en couche monocellulaire en présence d'un milieu à l'hydrolysat de lactalbumine dans des conditions précédemment décrites[4,5]. L'Arbovirus du groupe A utilisé (virus Sindbis, souche AR 339), entretenu sur cerveaux de souris, est introduit dans les cultures cellulaires à la 36e h, sous la forme de 3 ml d'une suspension contenant 5×10^3 P.F.U./ml, pour un tapis cellulaire de 15×10^6 cellules environ. Après 4 h de multiplication virale, les cellules sont récoltées par procédé mécanique, puis mises en suspension en tampon *Tris*-HCl 0,05 *M*, pH 7, saccharose 0,25 *M*, $MgCl_2$ 0,001 *M*. Les cellules sont ensuite broyées en milieu réfrigéré à l'aide d'un homogénéiseur (Ultra-Turrax TP 18/2), fonctionnant sous 220 V, pendant 40 sec en étapes fractionnées. Les noyaux, les mitochondries et les cellules non broyées sont sédimentés en 15 min à 10000 *g*. Les microsomes sont obtenus par centrifugation de 60 min à 198500 *g* et conservés par congélation à −193 °C.

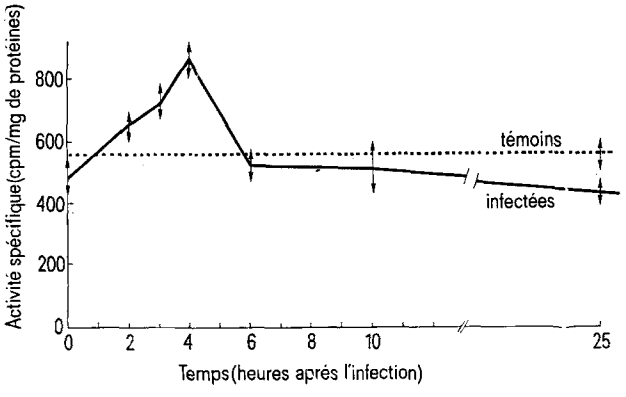

Fig. 1. Activité mannosyl-transférase microsomique en relation avec la durée de l'infection virale.

Les systèmes microsomiques de biosynthèses des glycoprotéines comprennent: 200 µl de la suspension de microsomes en tampon *Tris*-maléate 0,05 *M*, pH 6, contenant 2 à 3 mg de protéines par millilitre, dosées par la méthode de LOWRY et coll.[6]; 10 µl de $MnCl_2$ (concentration finale 5×10^{-3} *M*); 10 µl d'une solution contenant $1{,}3 \times 10^{-4}$ µmole de GDP-mannose ^{14}C (uniformément marqué, activité spécifique 154 µCi/µ*M*, NEN Corporation, USA). Les incubations sont réalisées à 30 °C. Au terme du temps de biosynthèse, les macromolécules glycoprotéiniques sont précipitées par addition d'acide trichloracétique (concentration finale p/v 10%) sur filtre en fibre de verre (Whatman Glass Paper, GF/B). Les précipités sont lavés par un mélange méthylal/méthanol (4/1), puis séchés pendant 1 h à 100 °C. La radioactivité est évaluée en flacons normalisés de comptage en présence

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