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Increase in the ileal absorption rate of sodium taurocholate in germ-free or conventional rats given an amylomaize-starch diet

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1. Twenty germ-free and twenty conventional male Fischer 344 rats, 3 months old, were fed *ad lib.* diets based either on normal or on amylomaize starch for 1 month. The absorption rate of sodium taurocholate, 25 mmol/l, was determined in vivo in jejunal or ileal segments. Each determination included five rats.

2. Jejunal absorption rate was not modified either by the amylomaize-based diet or by the digestive microflora.

3. Ileal absorption rate was slightly higher in germ-free than in conventional rats.

4. Ileal absorption rate was largely increased by the amylomaize-based diet. This increase was slightly larger in germ-free rats than in conventional rats.

5. It is suggested that dietary amylomaize starch has an action on the active absorption process of bile acid.

The presence of bile acids in the small intestine facilitates digestion and absorption of dietary lipids, cholesterol and fat-soluble vitamins. In man, patients with cholelithiasis have been found to have a diminished bile acid pool (Vlahcevic *et al.* 1970). However, in the experimental animal this pool, particularly the amount of bile acids in the small intestine, varies according to the presence or absence of microflora and according to various dietary factors (Wostmann 1973; Sacquet *et al.* 1979). Recently we have observed that the amount of bile acids in the small intestine of germ-free (GF) and conventional (CV) rats fed on a diet including amylomaize starch (E) was twice as large as that of GF and CV rats fed on a diet based on normal maize starch (MS) (Sacquet *et al.* 1983).

The question arises: how does amylomaize starch produce such an effect? It has been well established that during the enterohepatic circulation passive absorption of the bile acids takes place along the intestines, together with active absorption in the ileal segment. The latter might be the more efficient (Schiff *et al.* 1972; Riottot *et al.* 1975). Accordingly, it is possible to assume that if the absorption rate of bile acids increases in the distal part of the small intestine, the flow of bile through the bile duct is also increased. When the small intestinal transit time is not reduced or when it is increased, the amount of bile acids in this area becomes larger. A relation between the transit rate and the amount of bile acids in the small intestine has been observed in some cases (Riottot *et al.* 1980). An increase in the ileal absorption rate has also been observed in animals fed on a commercial chow as compared with a semi-synthetic diet (Riottot *et al.* 1975).

In the present study we assumed that the increased amount of bile acids in the small intestine of GF or CV rats receiving amylomaize starch is due to an increased absorption of these molecules. To verify this hypothesis, we compared the intestinal absorption rate of a physiological concentration of sodium taurocholate (NaTC) (Wostmann *et al.* 1976) in four groups of rats: GFE, CVE, GFMS and CVMS.

MATERIALS AND METHODS

Twenty GF and twenty CV Fischer 344 male rats were bred on a commercial diet until they were 3 months old. They then received a diet based on either maize starch (diet MS) or amylomaize starch (diet E) for 1 month before being submitted to the experiment. They

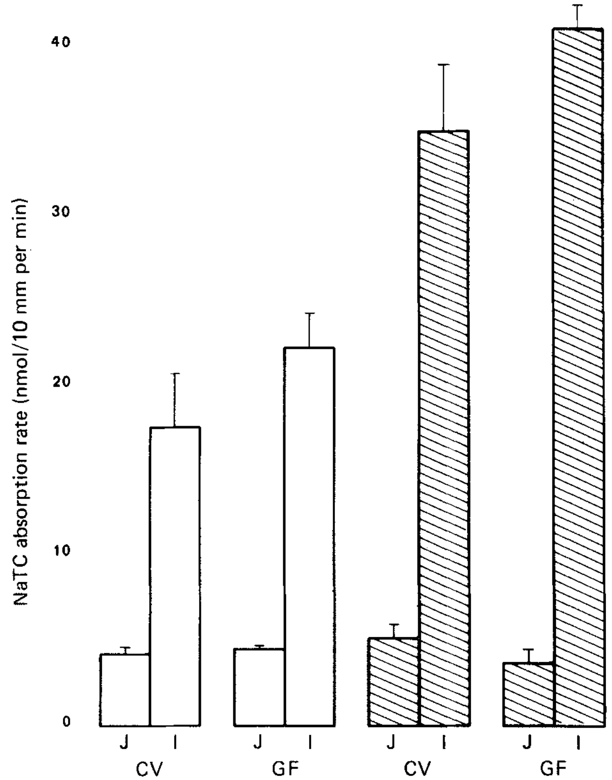

Fig. 1. Sodium taurocholate (NaTC) absorption rate (nmol/10 mm per min) in the jejunum (J) and in the ileum (I) of germ-free (GF) and conventional (CV) rats fed on either the amylomaize (▧) or the normal (□) starch diet. Values are means, with their standard errors represented by vertical bars, for five rats.

formed the four experimental groups: GFMS, GFE, CVMS, CVE with ten rats per group. The mean (with SE) live weights (g) of 4-month-old rats were: GFMS 291·7 (7·7), GFE 305 (7·8), CVMS 284·4 (7·4), CVE 260·7 (4·8). The experimental semi-synthetic diets had a common basis (g): maize starch 580, casein 220, maize oil 90, cellulose 50, mineral and vitamin mix 45 (Andrieux *et al.* 1980). The normal maize starch in diet MS contained (g/kg) amylose 300 and amylopectin 700, and the amylomaize starch in diet E contained (g/kg) amylose 700 and amylopectin 300 (Roquette Frères, 62136 Lestrem, France). Both diets MS and E were introduced in the form of a dry powder into polyethylene bags, sealed under vacuum, then sterilized by gamma irradiation at 40 kGy. They were mixed with water (1: 1, w/w) and offered to the rats in the form of a paste.

The absorption rate of NaTC (grade A; Calbiochem, CA) at a concentration of 25 mmol/l was measured using Schiff's technique (Schiff *et al.* 1972) modified by Riottot *et al.* (1975, 1977). Jejunal sections (200 mm) whose upper end was located 100 mm from the ligament of Treitz or ileal sections of the same length whose lower end was located 100 mm from the ileo-caecal valve, were perfused in situ with NaTC solution at a flow rate of 0·5 ml/min. This solution contained 24-^{14}C-labelled NaTC (Amersham International plc, Amersham, Bucks; CFA 501, batch 8) 45000 disintegrations/min, 25 μmol NaTC/ml in a buffer containing 5 mmol/l disodic phosphate, 5 mmol/l Tris, 115 mmol/l sodium chloride and adjusted to pH 7·00 with hydrochloric acid. The chemical and radiochemical purities of

NaTC were tested by thin-layer chromatography using Hofmann's (1964) system (isoamyl acetate – propionic acid – propanol – water (40:30:20:10, by vol.) and, after deconjugation of the bile salt and methylation of the free cholic acid, by descending thin-layer chromatography using Hofmann's (1964) system S VII (chloroform – acetone – methanol (70:25:5, by vol.)) on silica-gel plates. The rate of appearance of the labelled NaTC in the bile usually became linear with respect to time after 15 min of intestinal perfusion. This absorption rate was determined by dividing the radioactivity excreted in the bile by the specific activity of 24-^{14}C-labelled NaTC perfused in the jejunal or ileal loop. This rate was expressed as nmol absorbed on a per 10 mm intestine and per min basis. Results were compared with the two-way variance analysis (Snedecor & Cochran, 1957).

RESULTS

The results are presented in Fig. 1. The jejunal absorption rate of NaTC was not significantly affected by the bacterial flora or by the diets ingested by the rat. The ileal absorption rate of NaTC was six to eight times higher than the jejunal absorption rate in CV and GF rats whatever the ingested diet. In GF rats the ileal absorption rate of NaTC was 15–20% higher than in CV rats; the difference was highly significant ($P < 0.002$). In CVE and GFE rats the ileal absorption rate was two times higher than that of CVMS and GFMS rats respectively ($P < 0.0001$). The interaction between bacterial flora and diet was significant ($P < 0.002$) although it was of small amplitude.

DISCUSSION

The absence of significant variation in the jejunal absorption rate of NaTC as affected by the diets and the bacterial flora confirms our previous observations (Riottot *et al.* 1975, 1977). These low rates correspond to those found by Lack & Weiner (1973) and Schiff *et al.* (1972), but they are in contradiction with those of Sklan *et al.* (1976) and McClintock & Shiau (1983) who asserted that jejunal absorption of NaTC was higher than that in the ileum. According to Schiff *et al.* (1972), bile acid transport is passive in the whole intestine except in the ileum, where it is associated with active transport. We may speculate that in the ileum, as in the jejunum, the passive absorption was not modified and that the increase in the ileal absorption rate was only due to the increase in the active absorption rate. The doubled rate in the terminal ileum might explain why CVE and GFE rats exhibited twice as much bile acids in their small intestine as CVMS and GFMS rats, since a larger amount of bile acid was retained on the entero-hepatic cycle.

This action was mainly due to amylomaize starch since the effect of the bacterial flora was small. Digestibility of this starch by the CV or the GF rats was lower than that of maize starch (Sacquet *et al.* 1983). Amylomaize starch may behave like a dietary fibre as suggested by Fujita *et al.* (1982) and exert its action on the enterocyte which absorbs NaTC. This action may be either direct, i.e. the grain itself or its hydrolysis products acting on the enterocyte, or indirect, i.e. the lower digestibility of amylomaize starch leading to an increase in the digestive secretion by neuro-hormonal stimulation. According to Kay *et al.* (1980), dietary fibres alter the resistance of mucosal barriers by modifying the unstirred water layers. These factors change the passive absorption of solutions. In the present experiment, neither the very digestible maize starch nor the amylomaize starch modified the jejunal absorption of NaTC. As far as the jejunal absorption is passive, the results are inconsistent with the previously mentioned hypothesis. The action of amylomaize starch on the active absorption might result either from the increase in the number of absorption sites or from the increase in the amount or activity of the carrier. Further studies are still required to elucidate the major role of amylomaize starch in the active absorption of NaTC by the enterocyte.

The increase in ileal absorption is not the only mechanism liable to modify the bile acid contents of the small intestine. For example, the germ-free status increases the bile acid contents by two- or threefold without much effect on absorption (Riottot *et al.* 1975). The effect is probably to slow down small intestinal transit time (Abrams & Bishop, 1967; Sacquet *et al.* 1970). Some dietary factors have the same effect (Riottot *et al.* 1980, 1984). However, amylomaize starch is not the only substance capable of enlarging the bile acid pool of the intestine by increasing the ileal absorption. We have previously observed the same effect with a commercial chow which did not contain this starch (Sacquet *et al.* 1977). As this diet was sterilized by autoclaving, which denatures most of the starches, the active substance is probably not a starch. Therefore other dietary components might play a part similar to that of amylomaize starch.

The results may have some implications for human nutrition. According to Vlahcevic *et al.* (1970) the bile acid pool is reduced in cholelithiasis patients but the origin of this reduction had not yet been established. Factors other than dietary ones might be involved, such as hormones like thryoxin (Mathé & Chevallier 1981) or insulin (Nervi *et al.* 1974). Man never ingests semi-synthetic diets so that it would be more logical to say that it is the semi-synthetic diet compared with the complex diet which reduces the bile acid pool rather than the reverse. However, some components of complex diets might be capable of modifying this pool and they have to be determined.

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