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EFFECT OF WHEAT BRAN ON EXCRETION OF RADIOACTIVELY LABELED ESTRADIOL-17β AND ESTRONE-GLUCURONIDE INJECTED INTRAVENOUSLY IN MALE RATS

C. J. M. ARTS,[1]* C. A. R. L. GOVERS,[1] H. VAN DEN BERG,[1] M. A. BLANKENSTEIN[2] and J. H. H. THIJSSEN[2]

[1]TNO Toxicology and Nutrition Institute, Department of Experimental Biology, P.O. Box 360, 3700 AJ Zeist and [2]Academic Hospital, Department of Endocrinology, Utrecht, The Netherlands

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Summary—Urinary and fecal estrogen excretion were studied in male rats fed a non-fiber wheat starch diet (dietary fiber <1%; NF group; $n = 4$), a low-fiber wheat flour diet (dietary fiber 2%; LF group; $n = 4$) or a high-fiber wheat bran diet (dietary fiber 11.6%; HF group; $n = 3$). Short-term effects of the experimental diet on estrogen excretion were studied after i.v. injection of 5 μCi (0.185 MBq) of [^{14}C]estradiol-17β(E_2) into the tail vein of the rats fed the diets for 2 days. After 3 weeks on the experimental diets, the long-term effects were studied after injection of 5 μCi of [^{14}C]E_2 and 10 μCi of [^{3}H]estrone-3-glucuronide (E_1-gluc). The diet was found to affect estrogen excretion. The short-term effect indicated that rats fed the HF diet excreted a relatively large amount of labeled compounds in the feces during the first day after injection, while rats fed the NF or the LF diets excreted about half that amount over the same period. On the other hand, urinary excretion of labeled compounds was significantly higher in the NF and LF rats. The long-term effect resulted in steeper slopes ($P < 0.05$) of the fecal excretion profiles of rats fed the HF diet as compared with rats fed the NF and LF diets, indicating an accelerated fecal excretion of labeled compounds in the HF rats. The kinetic profiles of ^{14}C and ^{3}H radioactivity in blood plasma indicated a fast decrease ($t_{1/2}$ of <2 min) for both [^{14}C]E_2 and [^{3}H]E_1-gluc. It was concluded that, owing to the short-term effect of wheat bran intake, during the first 24 h after i.v. administration relatively large amounts of radioactively labeled compounds are excreted in feces of rats fed the HF diet. In contrast, excretion is lower in urine of these rats. When the microflora is adapted to the experimental diet the wheat bran diet still results in an accelerated fecal excretion of labeled compounds, which might be attributed to an interruption of the enterohepatic circulation of estrogens. This might result in lowered plasma and/or tissue estrogen levels and hence a decreased exposure of estrogen-sensitive tissue to estrogens, which might decrease risk on mammary (breast) cancer development.

INTRODUCTION

Estrogens have been reported to play a role in mammary carcinogenesis. An increased exposure of the estrogen-sensitive breast tissue would result in a higher risk of mammary cancer at the initiation and/or promotion phase [1]. Estrogens synthesized in the ovaries, and partly in the peripheral tissues, are mainly metabolized in the liver, and about 50% is excreted via the bile into the intestine. Subsequently, from the estrogens in the gastro–intestinal tract about 80% is reabsorbed via enterohepatic circulation [2].

Dietary fiber can partly interrupt the reabsorption of estrogens, resulting in an increased fecal and a decreased urinary estrogen excretion, and lowered plasma estrogen levels [3]. In rats fed a high-fiber (HF) diet based on wheat bran a 3-fold higher fecal estrogen excretion was measured than in rats fed a low-fiber (LF) diet based on white wheat flour [4]. The composition of the gut flora is influenced by dietary factors, in particular by indigestible plant cell wall materials [5]. This results in changed bacterial enzyme activities (lower β-glucuronidase, azo- and nitroreductase activity [6, 7]) after about 3 weeks on a HF diet [8]. Whether the increased

*To whom correspondence should be addressed.

Abbreviations: LF, low-fiber; HF, high-fiber; NF, non-fiber; HPLC, high performance liquid chromatography; i.v., intravenous(ly); BSA, bovine serum albumin; E_1, estrone; E_2, estradiol-17β; E_3, estriol; E_1-gluc, estrone-3-glucuronide; 16α-OH-E_1, 16α-hydroxyestrone.

fecal estrogen excretion is a result of binding to dietary fiber [9, 10], of a changed (intestinal) estrogen metabolism, or of both is not known.

In this report an experiment is described in which radioactively labeled estrogen excretion is studied in groups of male rats fed a non-fiber (NF) diet based on wheat starch, a LF diet based on white wheat flour, or a HF diet based on wheat bran. The study was performed to confirm the previous findings [4], i.e. an increased fecal estrogen excretion and a lowered urinary estrogen excretion by rats fed a HF diet, and to exclude a changed (increased) estrogen synthesis by intestinal microflora.

Short-term effects of feeding the different diets were studied by quantitating the disposal of [^{14}C]estradiol-17β([^{14}C]E_2) injected i.v. into the rats fed the LF and HF diets during two days. Long-term effects of intestinal estrogen metabolism were studied by injection of [^{14}C]E_2 and [^{3}H]estrone-glucuronide([^{3}H]E_1-gluc) to rats fed the experimental diets for 3 weeks, assuming intestinal flora is adapted by then [8, 11]. E_2 was used as the radioactively labeled hormone, because it is the biologically most active estrogen. E_1-gluc is a quantitatively important metabolite in urine. From the results it is concluded that a HF diet increased and accelerated fecal estrogen excretion and lowered urinary estrogen excretion.

MATERIALS AND METHODS

Animals and diets

Until the start of the experiment, male Wistar rats (Charles River Wiga, Fed. Rep. Germany; 13 weeks of age) were housed in groups of 4 animals each in an air-controlled room (23 ± 1°C) with a relative humidity of 50 ± 10% and a light/dark cycle of 12 h. Tap water and the NF diet based on wheat starch were administered *ad libitum* for 20 days. At the start of the experiment the rats were divided into three groups on the basis of body weight and housed individually in cages arranged in such a way that feces and urine could be collected separately. Group NF ($n = 4$) received the non-fiber diet based on wheat starch, group LF ($n = 6$) received the low-fiber diet based on white wheat flour and group HF ($n = 6$) the high-fiber diet based on wheat bran. The composition of the diets is given in Table 1. Water and the iso-energetically composed diets were administered *ad libitum*. Food intake was recorded weekly.

Table 1. Composition of the three diets (% w/w)

Ingredients	NF diet	LF diet	HF diet
Composition calculated			
Casein	22.53	22.53	15.69
Wheat starch	54.72	—	—
White wheat flour	—	54.72	42.00
Wheat bran	—	—	23.75
Mineral mixture	4.24	4.24	3.63
Vitamin ADEK prep.	0.36	0.36	0.31
Vitamin B mix	0.24	0.24	0.20
Lard	8.95	8.95	7.21
Sunflower oil	8.95	8.95	7.21
Composition analyzed			
Protein	21.3	27.1	23.2
Fat	17.7	18.4	16.0
Carbohydrate	48.4	41.3	38.5
Dietary fiber[a]	<1	2.0	11.6
Energy (kJ/100 g)	1831	1836	1634

[a]AOAC method [22].

Injection of labeled estrogens and collection of samples

After 2 days on the experimental diets 4 rats of each group were mildly anesthesized with ether and injected i.v. into the tail vein with 100 μl of a solution containing 5 μCi (0.185 MBq) of [4-^{14}C]E_2 (Amersham, 's-Hertogenbosch, Netherlands; sp. act. 56 mCi/mmol), 20% ethanol and 0.1% bovine serum albumin (BSA) in saline. The labeled steroids were checked on purity before use by HPLC ([^{14}C]E_2) or by solid-phase chromatography ([^{3}H]E_1-gluc; reversed-phase C18 cartridges; Baker Chemicals, Deventer, The Netherlands). One animal of the HF group died during anesthesia. During the following 7 days 24-h urine and feces were collected. Urine was collected on dry ice, thawed and the volume was measured; feces was weighed both wet and dry after lyophilization. All samples were stored at −20°C until further use.

After 20 days on the experimental diets all animals received a second injection with 200 μl of a solution containing 5 μCi of [^{14}C]E_2 and 10 μCi of [6,9-^{3}H]estrone-3-β-D-glucuronide (Amersham, 's-Hertogenbosch, The Netherlands; sp. act. 12.6 Ci/mmol). During the following 7 days, 24-h urine and feces were collected.

Two animals of the LF group and two animals of the HF group were equipped with a permanent indwelling catheter in the right external jugular vein. Via the catheter these animals were administered 200 μl of injection solution containing both 5 μCi [^{14}C]E_2 and 10 μCi [^{3}H]E_1-gluc. The cannules of the catheter were washed 3 times with saline containing 0.1% BSA, then 200 μl of blood was sampled 5, 10, 15, 30, 60, 120, 180 and 240 min after administration of the labeled estrogens. Blood samples

were transferred to Eppendorf cups containing 100 μl of 1.5% sodium citrate solution. After centrifugation the diluted plasma was stored at −20°C until further use.

Quantitation of radioactivity and separation of metabolites by HPLC

In each urine sample and in plasma samples total radioactivity (^{14}C or ^{3}H and ^{14}C) was measured over a 10-min period (counting error <1%) in a liquid scintillation counter (Wallac 1410, Pharmacia/LKB, Woerden, The Netherlands).

Lyophilized fecal samples were dismembrated (liquid nitrogen, 1 min, amplitude 12 mm) using a microdismembrator (B. Braun, Melsungen, Fed. Rep. Germany) and solubilized in 3 ml of a sodium acetate buffer pH 4.5. To 100 μl of the solution 2 ml of soluene (Packard Instrument, Groningen, The Netherlands) was added and samples were incubated for 48 h. Hydrogen peroxide was added and after incubation for 24 h, 10 ml of scintillation liquid (Hionic fluor, Packard Instrument) was added. Total radioactivity (^{14}C or ^{14}C and ^{3}H) was measured. Labeled compounds from the dismembrated fecal solution were extracted with diethyl ether before (free fraction) and after (conjugated fraction) hydrolysis with a solution containing 4500 Fishman U β-glucuronidase and 45000 Roy U sulphatase activities (Suc d'Helix Pomatia; Boehringer Mannheim, Almere, The Netherlands; 2 h, 50°C, pH 4.5). These extracts were used for counting radioactivity as well as separation of the labeled estrogen metabolites by HPLC.

HPLC was performed as described previously [12]. Briefly, a column (125 × 4.5 mm) was filled with Lichrosorb diol, the eluent being hexane–isopropanol (90:10, v/v), at a flow rate of 1.2 ml/min. Fractions of 0.5 min each were collected during a run of 20 min per sample. To each fraction scintillation liquid (Safefluor) was added and radioactivity was counted. A tentative identification of peaks of radioactivity of the samples was performed by comparing the elution times with elution times of authentic E_1, E_2, estriol (E_3) and 16α-hydroxyestrone (16α-OH-E_1), eluted under the same conditions as the samples.

β-Glucuronidase activity and pH of cecal and intestinal contents

At 7 days after the second injection the rats were killed by an excess of ether anesthesia. The cecal and intestinal contents were used for measuring β-glucuronidase activity and pH as described [4].

Statistics

Differences in fecal and urinary radioactivity excretion as well as differences in β-glucuronidase activity and pH were analyzed using Student's t-test. As slopes of excretion profiles were only calculated from straight lines after log transformation, these parameters were calculated from day 2 of the collection period to the time 0.1% of the radioactivity relative to the amount injected had been excreted. Slopes were calculated using BMDP program 3R [13], while clearance was calculated as 'ln 2/slope'. Slopes and clearance were compared among the groups by analysis of variance (ANOVA) techniques. A P-value of <0.05 was considered to reflect statistical significance.

RESULTS

Body wt and food intake

At the start of the experiment the body wt (mean ± SD) of the rats ($n = 4$ per group) were 344 ± 36, 347 ± 29 and 349 ± 29 g for the NF, LF and HF groups, respectively. Food and energy intake of the NF and LF groups were similar. A higher mass of food was consumed by the HF group and during the first week energy intake was higher ($P = 0.05$) for the HF group than for the LF and NF groups. Food intake of all three groups was lower during the first week, when animals had to acclimatize from group accommodation to individual housing, than during the second week of the experiment (Table 2).

Excretion of labeled compounds

After the first injection total urinary radioactivity excretion over the 7-day collection period was significantly ($P < 0.05$) lower in the HF group than in the LF and NF groups (Table 3). After the second injection, when the animals

Table 2. Food (g/day) and energy intake (kJ/day) by male rats (weighing about 350 g) fed a NF, LF or HF diet

	NF	LF	HF
Week 1			
g/day	10.0 ± 1.4[a]	9.9 ± 0.7	13.4 ± 1.2
kJ/day	182 ± 25.5	182 ± 12.6	218 ± 19.7*
Week 2			
g/day	13.9 ± 1.9	14.0 ± 0.8	16.8 ± 1.6
kJ/day	254 ± 35.7	257 ± 14.3	275 ± 26.4

*Different ($P = 0.05$) from the NF and LF groups.
[a]Mean ± SD.

Table 3. Excretion of ^{14}C- and ^{3}H-labeled compounds (% of total radioactivity injected; mean ± SD) in urinary and fecal samples collected for 7 days after injection

	Injection of $[^{14}C]E_2$			Injection of $[^{14}C]E_2$ and $[^{3}H]E_1$-gluc					
	NF	LF	HF	NF	LF	HF	NF	LF	HF
		% ^{14}C			% ^{14}C			% ^{3}H	
Urine	18 ± 7.2[a]	14 ± 3.4	8.8 ± 2.1	19 ± 4.0	22 ± 3.6	17 ± 4.1	18 ± 3.9	22 ± 4.0	17 ± 3.0
Feces	90 ± 25	55 ± 20	89 ± 16	76 ± 8.2	71 ± 5.3	71 ± 2.2	76 ± 6.4	72 ± 5.8	73 ± 2.9
Total	107	69	97	96	94	88	94	94	89

[a]Mean ± SD.

Injection of $[^{14}C]E_2$ was performed when rats had been fed the NF ($n = 4$), LF ($n = 4$) and HF ($n = 3$) diets for 2 days. Injection of $[^{14}C]E_2$ and $[^{3}H]E_1$-gluc was performed when rats had been fed the experimental diets for 3 weeks.

had been fed the experimental diets for 3 weeks, no difference in total urinary ^{14}C and ^{3}H radioactivity excretion among the groups was observed. Excretion of ^{14}C and ^{3}H radioactivity, expressed as percentage of total radioactivity injected, was similar. Total fecal ^{14}C excretion was similar for the NF and HF groups, but lower for the LF group, after the first injection and similar for all 3 groups after the second injection. Recovery of ^{14}C and ^{3}H radioactivity in urinary and fecal samples ranged between 88 and 108% for all groups, but total recovery of the LF group was only 69% after the first injection (Table 3).

The daily cumulative radioactivity excretion and the excretion profiles for ^{14}C after the first and after the second injection are presented in Figs 1 and 2, respectively. Because the figures for ^{3}H radioactivity excretion were very similar to those of the ^{14}C excretion, the results have not been shown separately. The relatively small amount of ^{14}C excreted in urine of the HF group after the first injection (Table 3) was excreted faster than in the other groups. The slope of the excretion profile of the HF group was steeper than for the other groups [$P < 0.05$; Fig. 2(A)].

Fecal ^{14}C excretion after the first injection varied largely among the groups (Figs 1 and 2). In the HF group, 73% of total ^{14}C fecal radioactivity excreted over the 5-day period was excreted during the first 24 h and 20% during the second 24-h period. In the other two groups about 30% of total fecal radioactivity was excreted during the first day and 42% (NF group)

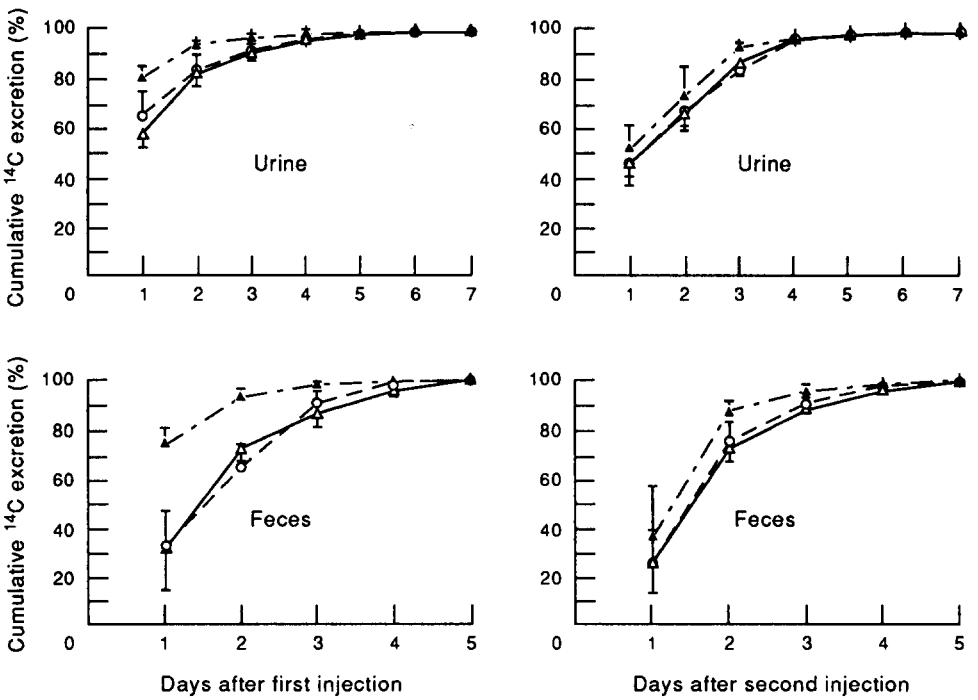

Fig. 1. Cumulative excretion pattern of ^{14}C radioactivity in urinary and fecal samples from rats fed 2 days or 3 weeks on a NF (wheat starch; —○—), LF; (wheat flour; —△—) or HF (wheat bran; —▲—). Rats were injected with 5 μCi $[^{14}C]E_2$. The marks indicate means and SD. 100% of radioactivity equals total mean excretion per rat and per group, *cf* Table 3.

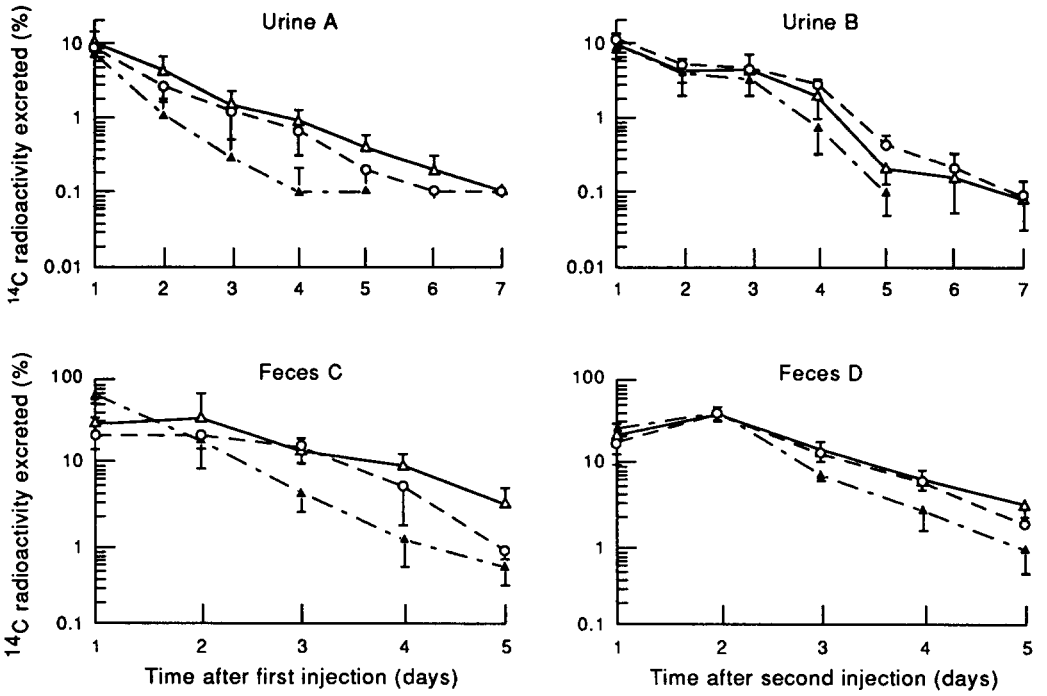

Fig. 2. Urinary (A,B) and fecal (C,D) excretion profiles of ^{14}C radioactivity after injection of 5 μCi [^{14}C]E_2 in rats fed the experimental diet for 2 days (A,C) or 3 weeks (B,C). NF, —○—; LF, —△—, HF, —▲—. The marks indicate means and SD. 100% of radioactivity equals total mean excretion per rat and per group, *cf* Table 3.

or 32% (LF group) during the second day after injection (Fig. 1). The excretion rate of the HF group tended to be higher ($t_{1/2} = 0.54$ days) than for the other groups ($t_{1/2}$ about 1.15 days). After 3 weeks the fecal excretion profiles of the NF and LF groups were similar and comparable with the excretion profiles of the NF group after the first injection. The HF group, however, showed a remarkably different fecal excretion profile after 3 weeks on the HF diet as compared with the profile obtained after the HF diet was administered during 2 days. A straight profile was obtained after 2 days on the HF diet, while after 3 weeks the excretion profile showed a higher fecal radioactivity excretion on the second day than on the first day (Fig. 2). Slopes of the fecal radioactivity profiles of the HF group were steeper ($P < 0.05$) than those of the other two groups.

Kinetics in blood plasma

Mean ^{3}H an ^{14}C radioactivity in blood plasma, sampled from 2 animals of both the LF and HF group, is given in Fig. 3. A fast decrease of radioactivity was observed between 5 and 15 min after injection. During the subsequent 15 min, ^{3}H radioactivity decreased to about 0.4 nCi/ml and ^{14}C radioactivity to about 1 nCi/ml. ^{14}C radioactivity remained fairly constant between 15 and 180 min after injection [Fig. 3(A)]. ^{3}H radioactivity increased slightly

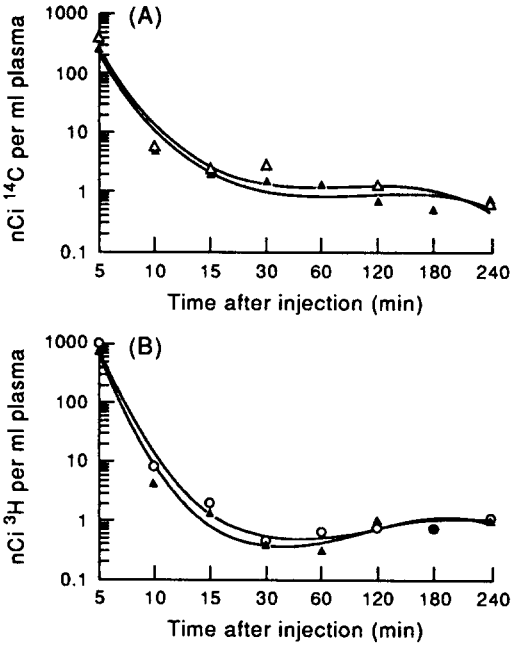

Fig. 3. Kinetic profile of ^{3}H and ^{14}C radioactivity in blood plasma of 2 animals of both the LF group (○) and the HF group (▲). (A) As a result of injection of 5 μCi [^{14}C]E_2, and (B) resulting from injection of 10 μCi [^{3}H]E_1-gluc.

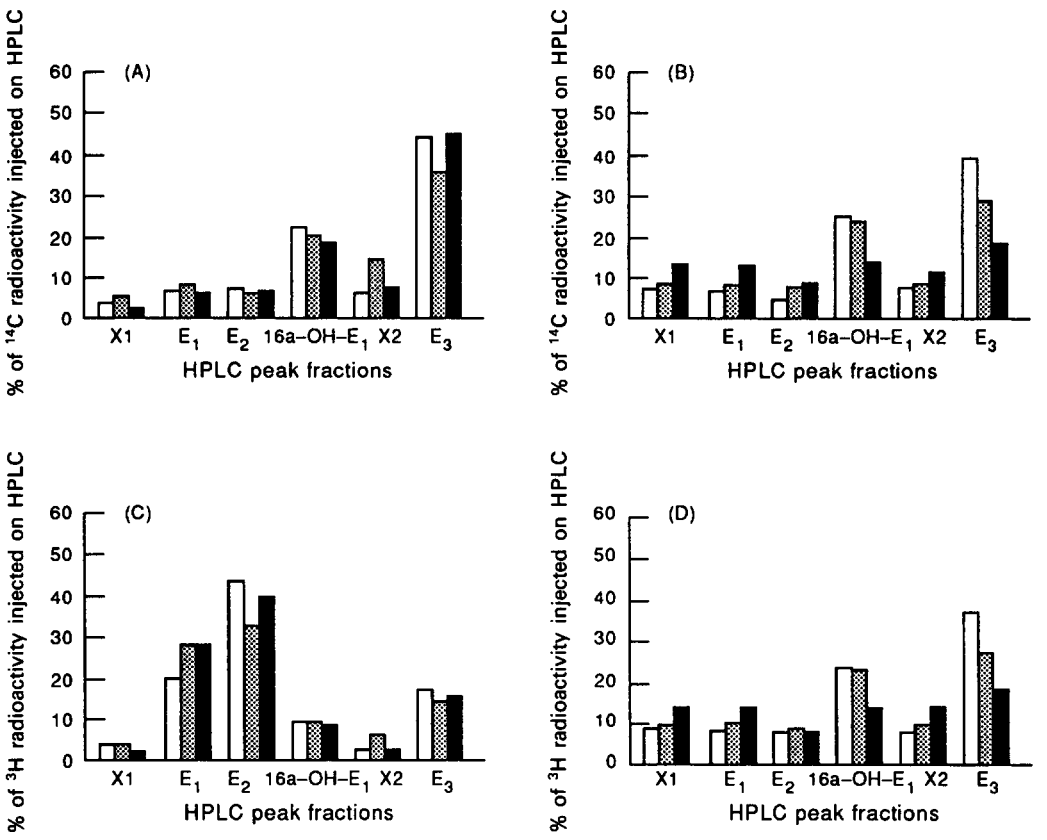

Fig. 4. Relative distribution of radioactivity among HPLC fractions: fecal samples collected 1 (A,C) and 3 days (B,D) after injections of 5 μCi of [^{14}C]E_2 (A,B) and 10 μCi of [^{3}H]E_1-gluc (C,D). HPLC fractions containing radioactive compounds with the same elution times as authentic E_1, E_2, E_3 and 16α-OH-E_1 were collected. Peaks indicated with X1 and X2 contain unknown compounds. Histograms: white, NF group; stippled, LF group; and solid, HF group.

between 60 and 180 min after injection [Fig. 3(B)], which might be the result of enterohepatic circulation or of a delayed release of the labeled compounds by the tissues (e.g. fat tissue).

Fecal estrogen metabolites

In the HPLC fractions obtained from feces collected during the first day after the second injection, ^{14}C [Fig. 4(A)] and ^{3}H radioactivity [Fig. 4(C)] gave different excretion profiles of estrogen metabolites. The metabolites 16α-OH-E_1 and E_3 were the major compounds in the HPLC profile after injection of [^{14}C]E_2, whereas E_1 and E_2 were the major compounds after injection of [^{3}H]E_1-gluc. Three days after the second injection no differences in the HPLC profiles could be observed between excretion of ^{14}C [Fig. 4(B)] and ^{3}H [Fig. 4(D)] compounds. Peaks with the highest radioactivity primarily derived from compounds that elute during HPLC as authentic 16α-OH-E_1 and E_3. In feces of the NF and LF groups about 12% of total ^{14}C or ^{3}H radioactivity injected was excreted during the third day, whereas in the HF group about 6% was excreted during this period. Furthermore, in the HF group the relative amount of labeled compounds with elution time as authentic 16α-OH-E_1 and E_3 was lower and as X_1 and authentic E_1 higher than in the NF and LF groups.

Fecal weights

As shown in Table 4, total fecal excretion (both wet and dry wt) for the HF group was 3 to 4 times higher than for the NF and LF groups. The higher fecal wet weight of the HF

Table 4. Mean fecal excretion (g/day)[a] by groups of rats fed a wheat starch NF ($n = 4$), a wheat flour LF ($n = 4$) or a wheat bran HF diet ($n = 3$)

Group	Wet wt	Dry wt	% moisture
NF	0.79 ± 0.35^a	0.52 ± 0.16^a	28 ± 7.5^a
LF	0.80 ± 0.21^a	0.63 ± 0.19^a	22 ± 10^a
HF	3.05 ± 0.34^b	1.97 ± 0.22^b	35 ± 4.1^a

[a]Mean fecal weights of samples collected 1, 6 and 7 days after the first injection.

Mean $\pm$ SD values sharing the same letter within a column are not significantly different ($P > 0.05$).

Table 5. Intestinal and cecal β-glucuronidase activity (U/kg) and pH in rats 7 days after the second injection (see text for more details) as well as the amount of ether extractable radioactivity (% free estrogen) as a percentage of radioactivity extracted before and after hydrolysis, from fecal samples of rats fed the experimental diets for 3 weeks

					Percentage of free estrogen (mean ± SD)					
	β-Glucuronidase (mean ± SD)		pH (mean ± SD)		Day 1*		Day 2		Day 3	
Groups	Intestine	Cecum	Intestine	Cecum	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
NF ($n = 4$)	41 ± 15^a	675 ± 177^a	6.5 ± 0.1^a	7.8 ± 0.2^a	60 ± 4.7^a	60 ± 5.7^a	66 ± 3.5^a	63 ± 3.8^a	43 ± 9.3^a	41 ± 9.1^a
LF ($n = 4$)	39 ± 14^a	1074 ± 242^b	6.7 ± 0.2^a	7.4 ± 0.1^b	54 ± 5.8^a	51 ± 6.7^a	55 ± 3.2^b	51 ± 4.7^a	43 ± 6.6^a	40 ± 6.1^a
HF ($n = 3$)	85 ± 21^b	608 ± 289^{ab}	6.9 ± 0.5^a	6.8 ± 0.2^c	53 ± 4.5^a	54 ± 2.3^a	58 ± 7.6^a	60 ± 5.0^a	60 ± 3.4^b	60 ± 2.8^b

*Remarks: Only the first 3-day samples collected were used for calculating % free estrogen, because in samples collected at later times after injection, radioactivity was too low for a reliable calculation. Mean ± SD values sharing the same letter within a row are not significantly different ($P > 0.05$).

group is only partly explained by the higher moisture content.

pH and β-glucuronidase activity

Mean cecal β-glucuronidase activity of the NF and HF groups was lower than for the LF group (Table 5). Cecal pH differed significantly among the groups, but pH of the intestinal contents did not.

In Table 5 the percentage of unconjugated (free) estrogen is also given, expressed as a percentage of ether-extractable radioactivity without hydrolysis. Percentages of free estrogen in feces collected during the first day were similar. During the second day, however, the percentage of free estrogen as a result of injection of [^{3}H]E_1-gluc was lower in the LF group than in the other two groups. During the third day the rats of the HF group showed a significantly higher percentage of free estrogen (as a result of injection of both [^{3}H]E_1-gluc and [^{14}C]E_2) than the animals of the NF and LF groups. The higher percentage of free estrogen in the HF group does not agree with the previous finding, i.e. a lower β-glucuronidase activity.

DISCUSSION

In our experiments differences in estrogen excretion as a result of ingestion of different diets were studied. To avoid possible effects of the estrous cycle on estrogen excretion, male rats were used instead of females, as the estrus cycle might influence intestinal motility [14] and therefore reabsorption and fecal estrogen excretion. A difference in estrogen excretion between male and female rats can be expected as estrogen metabolism concerning 16α-OH-E_1 synthesis has been found to be strongly sex-dependent [15]. Sex was not considered to influence any diet-induced differences.

Short-term effects

From the hypotheses stated previously [4,16, 17] it could be expected that a HF diet affects estrogen excretion resulting in an increased fecal estrogen excretion, a decreased urinary estrogen excretion and lower plasma estrogen levels. In the present study the short-term effect of a high-fiber intake indeed resulted in a significantly lower urinary ^{14}C excretion, while fecal ^{14}C excretion was 2- to 4-fold higher in the HF group than in the NF and LF groups during the first day after injection. The accelerated fecal excretion can be explained by interruption of the enterohepatic circulation due to binding of estrogens to dietary fiber components as demonstrated *in vitro* [9, 10]. It might be expected that during injection, as well as a few days after the first injection, the intestinal microflora of the animals fed the LF and HF diets was not changed relative to the microflora of the rats fed the NF diet which remained on the wheat starch diet [8]. For this reason, other explanations for the interrupted enterohepatic circulation than binding of estrogens to fiber might be excluded.

Long-term effects

The long-term effect, measured after 3 weeks on the experimental diets, resulted on the first day in a higher fecal excretion of labeled compounds in rats fed the HF diet and a steeper slope ($P < 0.05$) of the ^{14}C and ^{3}H excretion profiles of this group [Fig. 2(D)]. During the second 24-h period a higher fecal excretion of labeled compounds was measured in all 3 groups than during the first day. This suggests reabsorption of labeled estrogens after previous intestinal bile excretion during the first 24 h, i.e. enterohepatic circulation [3]. Thus fecal excretion of labeled compounds tended to be higher in the HF group, which confirms previous findings [4]. It also fits the hypothesis that

dietary fiber enhances fecal estrogen excretion resulting in a lowered exposition of estrogen to estrogen-sensitive tissue, and hence a reduced risk for mammary cancer development.

pH and β-glucuronidase

The intestinal flora may be expected to be adjusted to the experimental diets after 3 weeks [8, 11], resulting in a lower intestinal pH and lower β-glucuronidase activity in animals fed the HF diet. The results presented in Table 5 are in agreement with these expectations as well as with earlier findings [4] in which significantly lower β-glucuronidase activities were found in fecal samples from rats fed the HF diet than in rats fed the LF diet. The very low β-glucuronidase activity in the intestinal contents compared to that in cecal contents is in agreement with previous reports [18]. In the cecum from animals fed the HF diet a significantly lower pH was measured, and also cecal β-glucuronidase activity tended to be lower than in the LF group (Table 5). A lower β-glucuronidase activity might result in decreased deconjugation of the estrogens excreted by the bile and hence a lowered reabsorption by mucosal cells [2]. However, in feces from animals of the HF group collected on the third day a higher percentage of free (unconjugated) labeled compounds was found than in non-hydrolyzed samples of the NF and LF groups. This confirms the validity of suggestions discussed previously [4]. In our earlier study, significantly higher percentages of free (unconjugated) E_1 and E_2 were estimated in fecal samples from the HF group. This was explained by the higher pH of the LF fecal samples (less optimal pH for β-glucuronidase activity). It was suggested that other factors such as fecal bulk, transit time and binding capacity of the non-fermentable dietary fiber components, rather than β-glucuronidase activity as measured *in vitro* under standardized conditions determine fecal estrogen excretion.

Fecal estrogen metabolites

During the first day after injection of [^{3}H]E_1-gluc, E_1 and E_2 were the quantitatively most important metabolites excreted [Fig. 4(C)], whereas 16α-OH-E_1 and E_3 were dominant three days after injection [Fig. 4(D)]. This difference in excretion of estrogen metabolites was not observed after injection of [^{14}C]E_2 [*cf.* Fig. 4(A and B)]. It seems that the conversion of [^{3}H]E_1-gluc to 16α-OH-E_1 and E_3 takes more time than conversion to E_1 and E_3, in which process one or two enzymes are directly involved [19].

In a previous experiment [4] it was found that energy (i.e. fat) intake of a group of female rats fed a similar HF diet as used in this study, was 6–10% lower than for rats fed the LF diet. Although in this study a difference in fat intake between the groups was not measured during the experimental period (Table 2), it might be assumed that fat was utilized in a manner differently by rats fed the HF diet than by rats fed the LF diet. The relatively small amount of E_3 and 16α-OH-E_1 estimated in fecal samples from the HF group [*cf.* Fig. 4(B and D)] could thus be explained. Women consuming a low-fat diet and given radiolabeled E_2 (both orally and i.v.) excreted significantly lower levels of 16α-hydroxylated estrogens than women on a high-fat diet [20]. The 16α-OH-E_1 and E_3 levels in fecal samples from rats fed the HF diet are of special importance regarding the discussion on its carcinogenic properties [15].

In the HPLC fraction in which 16α-OH-E_1 eluted (Fig. 4), 16-keto-estradiol eluted as well. This estrogen metabolite can quantitatively be synthesized to the same extent as 16α-OH-E_1 [15]. In studies reviewed previously [2], 16-hydroxylated estrogens and E_3 were also found to be the main biliary metabolites of E_1 and E_2.

Blood plasma kinetics

The kinetic profiles of ^{14}C and ^{3}H radioactivity, shown in Fig. 3, indicate a fast decrease ($t_{1/2}$ of <2 min) for both [^{14}C]E_2 and [^{3}H]E_1-gluc. Differences between the LF and HF groups were not observed. This clearance rate supports earlier findings in which a similar clearance was estimated after injecting [^{3}H]E_2 into female Sprague–Dawley rats [21].

It can be concluded that, owing to the short-term effect of wheat bran intake, during the first 24-h period after i.v. administration a relatively large amount of radioactively labeled compounds is excreted in feces of rats fed the HF diet. In contrast, in urine of these rats excretion is lower. Once the microflora is adapted to the test diets the wheat bran diet still results in an accelerated fecal excretion of labeled compounds.

This effect of dietary fiber might result in a lowered estrogen exposition to estrogen-sensitive tissue and hence a lowered risk of mammary cancer at the initiation or promotion phase.

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