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STUDIES ON ANTINUTRITIVE COMPONNENTS OF THE RYE GRAIN. II. BALANCE AND METABOLISM OF 5-N-ALKYLRESORCINOLS IN RATS

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The excretion of alkylresorcinols ARs in faeces and urine of rats fed with diets containing wheat or rye bran on 3 different levels was studied. Additionaly the effect of wheat and rye bran extracted with acetone was examinated. It was shown that about 36% alkylresorcinols present in wheat diet and about 48% of these compounds present in rye diet were excreted in faeces in intact form. The linear regretion of intake against excreted ARs both of wheat or rye was observed. Chromatographic analysis showed the presence in urine of three distinct fractions of alk/en/ylresorcinol metabolites.

In 1967 Wieringa isolated from acetone extracts of rye a fraction which he regarded as responsible for making rye grain less nutritious than grain of other cereals. He performed his dietary experiments with rats and piglets, identifying the fraction reducing the apetite and impeding the growth of animals as 5-n-alkylresorcinols. It is still being disputed whether alkylresorcinols (ARs) are to some extent toxic [10, 11, 15] or not [2, 9, 12]. Kozubek's studies indicate that ARs act on cell membranes. His model in vitro experiments with erythrocytes [6-7] demonstrated that ARs in 25 μM/l concentration severely disrupt the structure and organization of cell membrane, leading to hemolysis of erythrocytes. The most active in this process were unsaturated and short-chain homologs of alkylresorcinols, i.e. forms which are most plentiful in rye.

No studies have so far been performed to determine whether ARs are absorbed entirely or only partially and what are the metabolic changes they undergo in animal organisms. Our present research is intended to fill this gap.

MATERIAL AND METHODS

The experiments (I and II) were performed with outbred Wistar rats, weighing 67-75 g, with five animals in each experimental group. In the preliminary four-day

period the animals were fed ad libitum, and in the subsequent five-day experimental period proper each rat received 10 g of diet per day. Throughout the experiment the animals were fed an isoprotein diet containing 9.4% protein. The composition of diets used in experiment I is given in Table 1; the diets with low and high ARs content (experiment II) are characterized in Table 2.

In order to remove ARs from rye bran, 1-kg portions of this material were placed in a separatory funnel and extracted with ca 4 l of acetone at solvent outflow rate of about 600 ml/h. After extraction, the bran was dried for two days on several layers of filter paper at room temperature. Untreated rye bran contained 4.2 mg ARs per gram; for wheat bran the figure was 2.1 mg/g. Following the acetone treatment the ARs contents were 0.24 and 0.12 mg/g, respectively. ARs content was determined by the method of Tłuścik et al. [13]. The standard was pentadecylresorcinol purified chromatographically in a column with silica gel.

ARs content was determined in lyophilized faeces samples. Every group fed containing various amounts of bran consisted of five animals, and determinations were made in individual samples. 1-g portions of lyophilized and powdered faeces were extracted for 24 h to 10 ml of a chloroform-methanol mixture (1 : 1 proportion), shaking occasionally. After the sediment settled, 25 µl portions of clear mixture were taken for analysis.

The ARs content in urine was determined as follows. During five days of the experiment, urine from individual rats was collected into Erlenmeyer flasks containing 20 ml of 2N sulphuric acid, following which the volume of the mixture was adjusted to 250 ml. One-fifth of the urine of each rat in each of the various experimental groups was collected and combined together. In each such sample, „averaged" for every animal group, we determined ARs and sought AR metabolites with thin-layer chromatography. In preparation for thin-layer chromatography, 20-ml urine samples were shaken with 6 ml of ethyl acetate and then centrifuged. The extraction with ethyl acetate was performed twice, and the combined extracts were vacuum evaporated till dry, dissolved in 0.2 ml ethyl acetate and subjected to chromatographic separation.

Homologs of ARs were determined according to Tłuścik and Kozubek [14]. Saturated and unsaturated ARs were determined by the method of Kaczmarek and Tłuścik [3]. Chromatographic separation was done on silica-gel-covered plates; the developing system for urine extracts was chloroform: ethyl acetate (1 : 1), and for faeces extracts chloroform: acetone (95 : 5). The chromatograms were developed by immersion in 0.1% solution of Fast Red B (diazonium salt colouring phenols in a specific way), in 1% acetic acid, or by spraying with an anisic aldehyde solution (0.5 ml anisic aldehyde + 10 ml acetic acid + 85 ml methanol + 5 ml concentrated sulphuric acid) and by heating the plates for 10-15 min at 110°C. This universal reagent gives the alkylated resorcinols a specific red colour.

Table 1. Composition of experimental diets with increasing amounts of rye and wheat bran (increasing content of ARs) in experiment I

Rat group	Untreated rye bran (%)	Untreated wheat bran (%)	Casein (%)	Mineral salts mixture (%)	Mixture water-soluble vitamins (%)	Vitamins A, D, E in oil (%)	Wheat starch	ARs content (mg) kg
I	65			4	1	1	29	2734
II	55.8		7.8	4	1	1	30.4	2343
III	42.9		22.1	4	1	1	30.0	1800
IV	32.2		26.6	4	1	1	35.2	1352
V		59.6	0.4	4	1	1	34.0	1251
VI		45.8	12.9	4	1	1	35.3	962
VII		34.2	26.0	4	1	1	33.8	718

Table 2. Composition of experimental diets in experiment II

Rat group	Rye bran	Wheat bran	Untreated (%)	Acetone-extracted (%)	Mineral salts mixture (%)	Mixture of water-soluble vitamins	Wheat starch	Soya oil with vitamins A, D, E (%)	ARs content (mg) kg
I	65				4	1	29	1	2734
II		65			4	1	29	1	156
III			65		4	1	29	1	1365
IV				65	4	1	29	1	77

Table 3. Absorption of AR in 5 days balance experiments in rats

Bran in diets %	Feed intake per 5 days g	AR intake mg/5 days	Mass of faeces per 5 days g	AR content in faeces mg/g	Total AR excreted in faeces mg/5 days	Absorbed AR % of intake
65.1 R	46.8±4.9	128.0±13.0	11.5±1.20	5.27±1.00	60.9±13.1	52.4
55.8 R	44.8±2.9	105.0± 6.7	8.6±1.90	5.11±0.52	43.1± 6.8	58.9
42.9 R	47.6±3.2	85.7± 5.6	7.0±0.91	4.67±0.55	32.4± 1.03	62.1
32.2 R	49.3±0.2	66.6± 0.7	6.4±0.90	3.54±0.48	22.4± 2.6	66.4
59.6 W	46.9±3.2	58.7± 4.0	9.8±0.65	1.80±0.17	17.7± 2.3	70.0
45.8 W	47.3±3.4	45.4± 3.2	7.6±1.02	1.76±0.16	13.3± 0.9	70.8
34.2 W	48.8±2.8	35.1± 1.7	6.4±0.53	1.29±0.19	8.2± 0.4	76.6

R — rye bran
W — wheat bran
± — standard deviation

RESULTS AND DISCUSSION

As can be seen from the obtained results (Table 3), an increase in the content of bran (i.e. also of ARs) in the diet is accompanied by an increase of excretion of ARs with faeces, up to 47.6% (including 128 mg of the studied compounds) in the case of the diet with 65.1% of rye bran over the five days of balance studies. The lowest percentage of excreted ARs (23.4%) was found in the diet with 34.2% of wheat bran; during five days of the experiment only 35.1 mg of ARs was recovered. The amount of excreted ARs does not depend on the kind and concentration of bran (wheat bran contains half the amount of ARs per unit volume than rye bran) but on the total intake of these compounds. This also indicates that ARs, which are phenolic lipides barely soluble in water, are completely emulgated from caryopsis involucres in the alimentary canal.

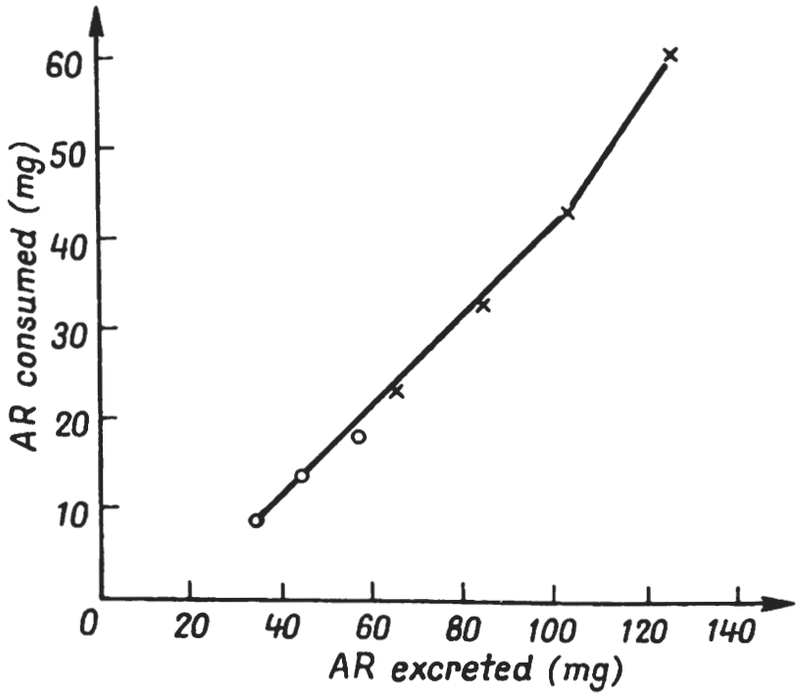

Fig. 1. Influence of AR intake on AR excretion in faeces; o — wheat bran diets, x — rye bran diets

The dependence between the intake and excretion of ARs is illustrated in Fig. 1. The straight-line dependence between these two values changes only for the highest intake (diet with 65.1% of rye bran). It is possible that excretion will rise sharply at still higher intakes. Standard deviation values (Table 3) also increase with the increase of bran content in the diet, being highest for 65.1% of rye bran. At this point, the variegated reaction of animals to such high contents of bran in the diet is already apparent. Basing on Fig. 1 we may write the following empirical formula for ARs excretion in faeces:

$$\text{Excreted ARs (in mg)} = \frac{\text{AR intake (in mg)} - 20 \text{ mg of AR}}{2}$$

As is known, after emulsification with bile acids salts and formation of micelles, amphophilic compounds are diffusion absorbed through the contact of micelles with the intestinal microvilli membrane [5]. It is thus possible that ARs,

given their amphophilic character, are contained in the micelles and diffuse from them to the villi membrane. As it turned out, however, only some of the ARs undergo diffusion. It is also known that a large amount in the diet of fibre and polysaccharides that are not decomposed by endoenzymes in the alimentary canal — i.e. of components which are plentiful in bran — favours the development in the intestines of bacterial flora capable of deconjugation of bile acids salts. Free bile acids do not form micelles effectively, and this may hinder the absorption of fats and also of ARs [1, 4].

Table 4. Percentage ratio of different alkyloresorcinol homologs in rat diets contaning rye (65%) or wheat bran (58%) and in faeces

Homolog	Rye		Wheat	
	diet	faeces	diet	faeces
Pentadecylresorcinol	5.6	4.0	4.1	3.8
Heptadecykresorcinol	27.0	24.2	11.0	10.1
Nonadecylresorcinol	33.8	34.0	34.0	35.0
Heneicosylresorcinol	18.9	20.0	33.2	35.1
Tricosylresorcinol	9.7	10.0	10.0	10.1
Pentacosylresorcinol	5.0	7.8	7.7	5.9
Summ	100.0	100.0	100.0	100.0

Table 5. Percentage ratio of saturated and unsaturated alkylresorcinols in rye and wheat bran diets and in faeces

	Rye		Wheat	
	diet	faeces	diet	faeces
Saturated alkylresorcinol %	78.1	79.0	91.8	92.4
Unsaturated alkylresorcinol %	29.1	21.0	8.2	7.6

Ward and Marquard [15] found that chicken fed with a rye-rich diet excreted more fat in faeces than chicken fed with wheat. The percentage of excreted fatty acids increases with the increase of the length of their chain, and decreases with the increase of their degree of unsaturation. In the case of our experiments concerning ARs, there were no significant changes in the excretion of the various AR homologs or of their forms with saturated or unsaturated side chains. The slight drop in the content of short-chain homologs, the richest in unsaturated chains, in the faeces extracts must be seen as due to their greater sensitivity to the procedure of preparing the material for analysis (Tables 4 and 5).

One must bear in mind that despite their amphophitic character, alkylresorcinols are nevertheless highly hydrophobic and this may very strongly limit micellization above a certain level that would enable the determination of differences in absorption of the various AR forms.

There is nothing to indicate that ARs undergo changes or degradation in the intestines due to bacterial flora. In thin-layer chromatograms coloured with Fast Red B and the anisic aldehyde solution there were no characteristically coloured fractions accompanying the AR fraction proper. This also means that there are no compounds in the faeces that would hinder quantitative determinations of ARs. Comparing the chromatographic fractions of extracts of urine from rats fed with

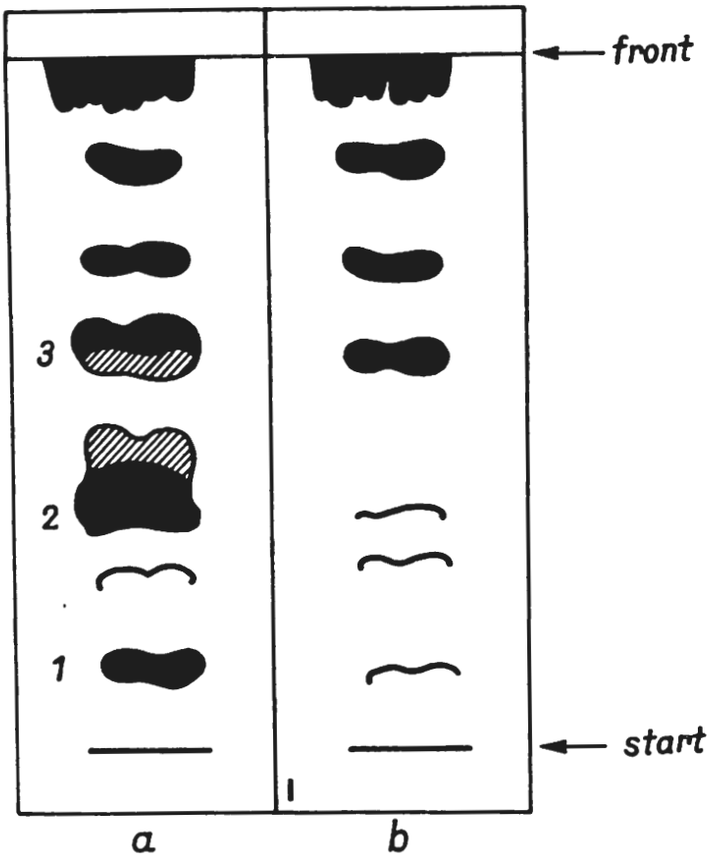

Fig. 2. Thin-layer chromatography of rat urine extracts, after feacing AR rich diet (65% row rye bran) or AR extracted diet (exp. II). Developing system-chloroform: ethyl acetate — 2:1. Separate spots were detected with aqueous Fast Red B (0.1%) a — rats fed with diet containing nonmodified rye or wheat bran, b — rats fed with diet containing alkylresorcinol free bran

untreated bran and with acetone-extracted bran (Fig. 2), we may assume without much risk of error that fractions 1-3 are AR metabolites. Further analyses concentrated on fraction 2 which coloured most intensely with Fast Red B. Using chromatography on aluminium oxide [14], we found that this fraction does not have homologs, and that the compound making it up does not have a long side chain. We failed to purify this fraction by rechromatography, among other things because insufficient amounts of urine were collected in the experiment. After rechromatography, the fraction gives several derivatives poorly stained with Fast Red B, with the principal fraction largely disappearing. It was tentatively determined that this principal metabolite is of a more polar character which

indicates the presence of an additional polar group directly at the resorcinol ring or on the short side chain. Evidence of this is the Rf value which is much lower than for ARs.

We are still working to identify the AR metabolites excreted with urine hoping to provide a fuller picture of the balance and fate of alkylresorcinols in the organism.

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STUDIA NAD ANTYODŻYWCZYMI SKŁADNIKAMI ZIARNA ŻYTA. II. BILANS I METABOLIZM 5-N-ALKILOREZORCYNOLI U SZCZURÓW

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Streszczenie

Przeanalizowano wchłanianie AR żyta i pszenicy (o różnej zawartości krótkołańcuchowych homologów) w bilansowych doświadczeniach na szczurach, którym podawano otręby żytnie (4,2 mg/AR/g) lub pszenne (2,1 mg AR/g) w ilości od ok. 30 do 65% diety. Oceniono również wydalanie AR w postaci niezmienionej w kale oraz metabolitów AR w moczu. Stwierdzono, że procent wchłaniania AR, zarówno żyta jak i pszenicy, spada liniowo wraz ze wzrostem spożycia AR, niezależnie od różnic zawartych w nim homologów. W kale wydalany jest AR w postaci niezmienionej, natomiast w moczu, jak wykazano w chromatografii cienkowarstwowej na tlenku glinu stwierdzono obecność 3 różnych metabolitów.

Kontynuowane są badania nad ich identyfikacją.