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The effect of a single oral dose of polyphenols obtained from the outercoat of the fruit of *Treculia africana* in protein-deficient rats

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The effect of an oral dose of 40 mg polyphenol kg^{-1} in protein-deficient male Wistar rats following a 5- or 10-day repletion regimen (RR) was investigated. There were increases in the body and liver weights of rats in test animals in comparison to well-nourished controls. In addition, urinary excretion of an oral dose of 40 mg polyphenol kg^{-1} was significantly less ($p < 0.05$) in chronically protein-dependent animals than other protein-malnourished counterparts. Excretion rates after 20 days were similar in all protein-deprived groups.

INTRODUCTION

The effect of diet-nutritional status, which is universally known to influence the metabolism and disposition of foreign chemicals, has been investigated by many workers (McLean & Versehuvren, 1969; Swann & McLean, 1971; Maduagwu, 1989). Feeding adult rats a protein-free or low protein diet protects against the acute toxicity of foreign compounds (Swann & McLean, 1971; McLean & Day, 1974). The metabolism of polyphenols takes place predominantly in the liver (Heath & Dutton, 1958; De Muelenaere, 1965; Einhellig, 1986). Polyphenols are known to be the most abundant secondary compounds of higher plants, exerting a wide range of biological effects in animals (Lowry & Sumpter, 1990).

The present work was undertaken to investigate the effect of a single oral dose of polyphenol obtained from the outercoat of the fruit of *Treculia africana* in protein-malnourished weanling rats following adequate dietary protein repletion of the animals.

MATERIALS AND METHODS

Polyphenol extraction from the outercoat of the fruit of *Treculia africana*

The outercoat of the fruit of *Treculia africana* was removed using a sharp knife, and 150 g was pounded in a mortar into a homogeneous slurry. The slurry was macerated in 300 ml of ethanol/water (50:50, v/v) with continuous shaking. After 24 h, the extract was filtered, and the residue subjected to a second maceration of 30 min and filtered. The filtrates were combined, concentrated by rotary evaporation at 50°C, centrifuged at 15000 rev. min^{-1} for 20 min, and the supernatant made up to 100 ml with distilled water. The extract was then freeze-dried.

Preparation of animals and diets

Male weanling albino Wistar rats (30–35 g), obtained from the Veterinary Science Faculty of the University of Ibadan, were divided into four groups of 10 rats.

Group A was fed, for 35 days, a low-protein diet made up of 3·5% protein (dietary casein), 81·5% carbohydrate (corn starch), 8% fat (vegetable oil), 4% salt mixture and 3% all-vitamin supplement (Boyd & Carsky, 1969).

Group B was given, for 7 days, a protein-free diet composed of 65% corn starch, 25% sucrose, 5% vegetable oil, 4% salt mixture and 1% vitamin mixture (Cuthbertson, 1957).

Group C was fed the low protein (3·5%) diet, also for 7 days only.

Group D the control, was placed on a protein-rich diet containing 20% protein (dietary casein), 65% carbohydrate (corn starch), 5% fat (vegetable oil), 4% salt mixture and 3% all-vitamin supplement for 35 days.

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Essentially, group A animals could be classified as chronically protein-dependent whereas the other experimental groups (B and C) were more acutely deprived models. All animals were fed and given drinking water *ad libitum*. Diet was compounded on a dry weight basis.

Protein-repletion experiments

The rats were placed *ad libitum* on the 20% protein regimen, which was also fed to the control animals in group D. The protein-repletion process was terminated after 5 days and 10 days respectively. Urinary excretion of an oral dose of 40 mg polyphenols kg^{-1} was investigated. The polyphenols were administered to the test animals using a stomach tube. At least 10 rats were used for each experiment. After 5 days and 10 days, respectively, a group of 10 rats each were given oral doses of 40 mg polyphenols kg^{-1}. Quantification of polyphenols was by a gas chromatographic method. Some of the animals (eight rats each) were decapitated on the 7th and 12th days, followed by blotting, discarding of extraneous tissue and washing the liver free of blood in ice-cold 0·15 M KCl.

Statistical analysis

The Student '*t*' test was employed in all statistical analysis.

RESULTS

Table 1 shows the effect of protein-repletion diet on body and liver weights of rats. In all cases, an increase in value was observed in test animals in comparison to well-nourished controls.

Table 2 shows the results for the *in vivo* degradation of polyphenols. Within 5 days of protein rehabilitation, the chronically protein-dependent rats excreted significantly ($p < 0.05$) less polyphenols in their urine than their other protein-malnourished counterparts. Excretion rates after 10 days were similar in all protein-malnourished animal groups. Non-appearance of polyphenols in the urine might be assumed to mean that they have been metabolized.

DISCUSSION

The results clearly show that dietary protein repletion enhances the metabolism of polyphenols. This may be

Table 1. Average changes in weights[a] of body and liver from day 0 to day 10, in protein-deficient male Wistar rats following process of repletion

Parameter	Protein-deficient animals		Protein-free	Well-nourished controls
	Chronically low protein	Acutely low protein		
Body weight (g)	46–86 (+40)	73–95 (+22)	70–105 (+35)	120–140 (+20)
Liver weight (g)	2·5–4·6 (+2·1)	3·9–5·1 (+1·2)	4·3–5·8 (+1·5)	5·9–7·3 (+1·4)
Ratio of liver to body	0·05	0·054	0·058	0·05

[a] Final weights (for four determinations).

Table 2. Urinary excretion of a single oral dose of 40 mg polyphenol kg^{-1} in protein-deficient male Wistar rats following a 5- or 10-day repletion regimen (RR)

Nutritional status of animal	Average[a] weight of animals (g)	Polyphenol excretion after 5-day RR (absolute mg)	Polyphenol excretion after 10-day RR (absolute mg)
Chronically low protein[b] (animals fed for 35 days)	49 ± 1·1	0·0008	0·0030
Acutely low protein[b] (animals fed for 7 days)	76 ± 1·5	0·0025	0·0036
Protein-free[b] (animals fed for 7 days)	65 ± 1·6	0·0017	0·0028
Well-nourished controls[b] (animals fed for 35 days)	175 ± 2·5	0·0061	0·0063

[a] Mean weights of four determinations ± standard deviation.
[b] There were eight animals in each group.

expected since the microsomal enzyme system in the liver, which deals with foreign compounds, chemicals and drugs, is known to fall to a low level during feeding of a protein-free diet or one low in protein content (McLean & McLean, 1966; Mgbodile & Campbell, 1972; Hayes & Campbell, 1974). Thus, as a result of dietary protein rehabilitation, the microsomal enzyme system seems to readjust itself.

Maduagwu (1989) showed that liver tissue which is chronically deficient in protein, synthesizes drug-metabolizing enzymes much more rapidly than tissue acutely deprived of protein, following protein repletion.

The greater increase in liver size and overall body weight noted in the chronically protein-dependent animals, within 10 days of protein repletion, in comparison to the other experimental groups (Table 1) agreed with the findings of Dijkstra (1964).

Toxicologically, the chronically protein-dependent weanling rats, in contrast to the acutely low-protein or protein-free animals, can be considered as an adapted species as a result of being chronically deprived of protein. Therefore, their marked response to protein repletion, in terms of polyphenol metabolism, seems to reflect an intrinsic difference in the effect of nutritional status on the metabolism of xenobiotics as distinct from dietary effects.

Dickerson *et al.* (1976) findings that in weanling but not in adult rats a low-protein diet includes an adaptive response in some of the hepatic drug-metabolizing enzymes, might correspond with the interpretation of our results in terms of an 'adaptive response' in the chronically protein-depleted rats (Table 2).

Further work on the metabolism of polyphenols obtained from the outercoat *in vivo* and *in vitro* will be reported later.

CONCLUSIONS

The effect of the protein-repletion diet on body and liver weights of rats showed increases in values in test animals in comparison to well-nourished controls.

Within 5 days of protein rehabilitation the chronically protein-dependent rats excreted significantly ($p < 0{\cdot}05$) less polyphenols in their urine than other protein-malnourished counterparts.

Excretion rates after 10 days protein rehabilitation were similar in all protein-deficient groups.

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