Bull. Environ. Contam. Toxicol. (1989) 42:294–300

Bulletin of Environmental Contamination and Toxicology

Effect of Petroleum Vapors Inhalation on Intestinal Absorption of Glucose and Some Amino Acids in the Rat

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The proper intestinal absorption of nutrients, particularly sugars and amino acids, is necessary to keep the organism healthy.

It is well known that various toxic compounds present in the environment can have an unfavorable influence. For example, pesticides like DDT (Mahmood 1978, Dudeja 1982), dieldrin (Reyman 1983), endosulfan (Wali 1982) and mycotoxins (Suneja 1984) alter the intestinal absorption of sugars and amino acids.

On the other hand it is also known that crude oil which pollutes the aqueous environment affects birds' gastrointestinal tract. Single oral doses of crude oil given to herring gull chicks affected growth, impaired osmoregulation and caused hypertrophy of the liver and adrenal and nasal glands (Miller et al. 1978). Chronic ingestion of crude oil caused histopathological changes in liver and kidneys of mallard ducklings (Szaro et al. 1978). In adult birds chronic ingestion of oil affected growth and modified hepatic function (Patton and Dieter 1980). Crocker and coworkers (1974, 1975) found that small oral doses of crude oil reduced Na^+ and water transport in intestinal section from saltwater-adapted ducklings. Eastin and Murray (1981) studied this problem with ducklings on a freshwater regime and they found out that crude oil didn't have any influence on the absorption of Na^+, Cl^-, K^+ or water, but paraffin significantly depressed Na^+ and water absorption.

Little is known about the influence of petroleum vapors on the gastrointestinal tract of animals and humans.

The present study was undertaken to determine the effect of petroleum vapors inhalation on intestinal absorption of some nutrients (glucose, leucine, methionine) in rats.

MATERIALS and METHODS

5 weeks old male Wistar rats with body weights ranging from 50 to 60 g were used in all experiments. They were fed a standard laboratory diet and received water *ad libitum.*

In the experiment the rats were randomly assigned to two groups, each group was divided into 5 small ones. Rats inhaled vapors of the light fraction of petroleum (10 g/m^3 or 30 g/m^3). The duration of the inhalation was different in each group : three days, six times a week for 1, 2 and 3 weeks, respectively. Control animals weren't subjected to inhalation.

After the inhalation period the animals were weighed and killed by decapitation. The jejunum was removed, blood samples were collected in heparinized beakers and immediately centrifuged.

Transport of glucose and amino acids in rat jejunum was studied *in vitro* by the method of everted gut sacs according to Wilson and Wiseman (1954).

After decapitation, the jejunum (8 cm) proximal to the duodenum (5 cm) was freed from the mesenteric attachments, isolated, everted on a stainless steel rod and thoroughly washed in cold 0.9% NaCl. The jejunum sacs were filled with 0.5 ml of Krebs-Ringer buffer solution containing 118.0 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and adjusted to a pH 7.4. The buffer contained also 5 mM D-glucose or 5 mM L-leucine or 5 mM L-methionine with trace amounts of ^{14}C-glucose or ^{14}C-leucine or ^{14}C-methionine. Next the sacs were placed in an Erlenmeyer flask containing 5.0 ml of the same buffer. The medium was saturated with oxygen and the flasks were shaken at 100 strokes/min in metabolic shaker at 37°C for 30 min.

After an incubation period 0.2 ml of mucosal (outer) and 0.2 ml of serosal (inner) fluids were taken for the total ^{14}C-glucose or ^{14}C-leucine or ^{14}C-methionine measurements. The samples containing the fluids were filled with 5.0 ml of dioxane fluid (Bray 1969). The radioactivity of samples was measured with an ISOCAP 300 liquid scintillation spectrometer.

The transport of nutrients was expressed as the percentage of the total applied dose of ^{14}C-glucose or ^{14}C-leucine or ^{14}C-methionine which had been transferred from the mucosal to the serosal side of the jejunal sac.

For the measurement of enzyme activity the mucosa was scraped from the jejunal portion of the gut (10 cm) using a glass slide and a 5% (w/v) homo - genate was made in 50 mM sodium maleate buffer (pH 6.5) The homogenate was centrifuged at 1,000 x g for 10 min at 4°C, the supernatant was used for the assay of enzymes activity.

Brush border sucrase, lactase and maltase were assayed by measuring glucose liberated from the respective substrates with a glucose oxidase and peroxidase system (Dahlqvist 1964). Leucine amino peptidase was assayed by the method of Goldbarg (1958).

For the estimation of Na^+K^+ATPase a 5% homogenate of the mucosal scrapings was made in 5 mM EDTA - 1 mM Tris-HCl, pH 7.4. It was centrifuged at 700 x g for 10 min and the supernatant was recentrifuged at 10,000 x g for 10 min

Table 1. The intestinal transport of ^{14}C-glucose and glucose levels in serum in rats after petroleum vapors inhalation

Concentration of petroleum vapors (g/m^3)	Time of inhalation	^{14}C-glucose transport (% ± SD)		Glucose level in serum (mg/100 ml)	
		Untreated (n)	Treated (n)	Untreated (n)	Treated (n)
10	3 weeks	9.72 ± 0.56 (6)	10.69 ± 0.43 (5)	76 ± 3 (7)	72 ± 3 (7)
30	3 days	11.09 ± 0.59 (6)	14.07 ± 0.45^b(5)	118 ± 5 (7)	101 ± 3^a (6)
30	1 week	11.25 ± 0.50 (6)	13.06 ± 0.68 (6)	87 ± 4 (6)	136 ± 3^b (7)
30	2 weeks	8.27 ± 0.50 (6)	9.58 ± 0.47 (7)	110 ± 1 (7)	96 ± 1^b (7)
30	3 weeks	9.19 ± 1.08 (5)	20.38 ± 1.04^b(9)	93 ± 1 (9)	77 ± 1^b (9)

Table 2. The intestinal transport of ^{14}C-leucine and ^{14}C-methionine in rats after petroleum vapors inhalation

Concentration of petroleum vapors (g/m^3)	Time of inhalation	^{14}C-leucine transport (% ± SD)		^{14}C-methionine transport (% ± SD)	
		Untreated (n)	Treated (n)	Untreated (n)	Treated (n)
10	3 weeks	5.81 ± 0.17 (7)	5.59 ± 0.25 (7)	8.62 ± 0.54 (6)	12.86 ± 0.52^b(7)
30	3 days	4.19 ± 0.16 (6)	6.48 ± 0.35^b(8)	7.02 ± 0.72 (6)	12.92 ± 0.62^b(6)
30	1 week	6.23 ± 0.45 (7)	12.81 ± 0.33^b(5)	8.10 ± 0.56 (6)	13.01 ± 0.58^b(8)
30	2 weeks	7.02 ± 0.49 (7)	12.40 ± 1.09^b(6)	8.06 ± 0.65 (7)	8.50 ± 0.57 (4)
30	3 weeks	9.65 ± 0.44 (6)	13.52 ± 0.75^b(7)	11.04 ± 1.34 (5)	14.59 ± 0.60^a(6)

a $P<0.05$ b $P<0.01$
n - number of observations

Table 3. Effect of petroleum vapors on some intestinal membrane bound enzymes.

Enzyme (unit)	Untreated	Treated
sucrase (U/mg protein)	4.27 ± 0.15 (6)	3.42 ± 0.11[b] (10)
maltase (U/mg protein)	2.45 ± 0.12 (6)	1.54 ± 0.13[b] (5)
lactase (U/mg protein)	0.25 ± 0.01 (6)	0.11 ± 0.02[b] (5)
leucine amino peptidase (U/mg protein)	0.12 ± 0.02 (7)	0.07 ± 0.01[a] (5)
Na^+K^+ATPase (μmol/h/mg protein)	0.06 ± 0.01 (7)	0.04 ± 0.00[a] (5)

a $P<0.05$ b $P<0.01$

at 4°C. The sediment was resuspended in 2.5 mM EDTA and the enzyme activity was assayed by measuring liberated phosphorus in a reaction mixture containing 5 mM ATP, 100 mM NaCl, 10 mM KCl (pH 7.0) during 30 min of incubation at 37°C.

All enzyme activities were calculated as units/mg protein. One enzyme unit is equal to 1 μmol of substrate transformed into product per minute under standard conditions. Protein was estimated by the method of Lowry (1951) using a bovine serum albumin as the standard. Serum glucose was determined by the method of Hywarinen (1962) using o-toluidine . Statistical analysis of the data was done using Student's t-test.

RESULTS and DISCUSSION

The results of glucose absorption across the rat jejunum after petroleum vapors intoxication are shown in Table 1. In rats inhaled at 30 g/m^3 petroleum, glucose transport was significantly increased after 3 days and 3 weeks of exposure (by 27 and 122%, respectively). Petroleum vapors at concentration 10 g/m^3 for 3 weeks didn't produce any changes in intestinal glucose transport.

The results presented in Table 2 indicate that the transport of leucine was significantly elevated in animals after 3 days and 1, 2 and 3 weeks inhalation at 30 g/m^3 petroleum - 55, 106, 77 and 40% above controls , respectively, but wasn't altered after 3 weeks of rats exposure at 10 g/m^3 petroleum.

Methionine transport in the gut also rose in rats subjected to petroleum vapors. After 3 days, 1 and 3 weeks of the inhalation (30 g/m^3) it increased to 184, 161 and 132% of controls, respectively, and it was also elevated after 3

Table 4. The body weight of rats during the exposure to petroleum vapors (g ± SD).

Untreated		Treated	
initially	after 3 weeks	initially	after 3 weeks
56 ± 2 (6)	146 ± 3 (5)	55 ± 2 (9)	87 ± 2 (7)
60 ± 2 (8)	156 ± 3 (8)	56 ± 2 (12)	86 ± 4 (12)

weeks of inhalation at 10 g/m^3 of petroleum vapors by 44%, as compared with controls.

The activities of brush border and basal lateral membrane enzymes are shown in Table 3. The activity of sucrase, maltase, lactase, leucine amino peptidase and Na^+K^+ATPase was diminished after inhalation at 30 g/m^3 petroleum vapors for 3 weeks - by 20, 37,, 55, 42 and 31%, respectively, as compared with controls.

There were no differences in protein concentration in serum between the control animals and the petroleum treated ones, but glucose level was reduced after 3 days, 2 and 3 weeks of inhalation at 30 g/m^3, by 14, 13, 17%, respectively (Table 1).

The body weight of untreated animals increased from 58 ± 2 g to 151 ± 3 g after 3 weeks. During the same time petroleum treated rats showed an increase of their body weight, but at the end of the experiment those animals weighed less than the untreated ones. Their weight amounted to 58% of the body weight of control group (Table 4).

The experiments described in the present report demonstrate an impairment of glucose and amino acids absorption in rat intestine as a consequence of petroleum inhalation. The studies also showed the inhibitory effect of petroleum hydrocarbons on the intestinal enzymes.

There exists a close functional relationship between the disaccharidases and the carrier-mediated sugar transport system in brush borders (Ramaswamy et al. 1976). A similar functional link between the peptidase activity and amino acids transport systems in intestine has also been demonstrated (Mathews 1972). Na^+K^+ATPase is required for maintaining the Na^+ gradient across the intestinal epithelial cell and crucial for the absorption of sugars and amino acids (Skou 1965).

Miller and coworkers (1978) showed that oral petroleum intoxication to birds caused ATPase inhibition, but in their experiment nutrient absorption was also diminished. Elevated absorption of sugars and amino acids was observed in pesticide - DDT (Mahmood et al. 1978,, Dudeja et al. 1982), dieldrin (Reyman

et al. 1983) and endosulfan (Wali et al. 1984) intoxication, which was accompanied by the stimulation of brush border enzymes.

The lack of correlation between sugar and amino acids intestinal transport, and the activity of brush border and basal lateral membrane enzymes observed in this experiment is difficult to explain. It may only testify to damage of membrane structure by liphophilic components of petroleum.

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