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EFFECTS OF BENZENE ON GLYCOGEN LEVELS OF LIVER AND MUSCLE TISSUES AND ON BLOOD GLUCOSE OF RATS

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Benzene is used commonly in industry and known as a toxic and carcinogenic agent. In this study a 100 mg.kg^{-1} dose was administered to Swiss Albino (Rat rattus norvegicus) rats by intraperitoneal injection. Changes in glycogen levels in the liver, muscle and blood glucose levels were investigated after 0, 2, 4, 8, 16, 32 and 64 hours.

In this study increased glycogen levels in liver and muscle tissues of both control and benzene-treated rats were found to depend on nourishment. The toxic effect of benzene disappeared at 64 hours after treatment. There was no significant difference between male and female groups regarding glucose levels except at a few time intervals.

In conclusion, our results indicate that glycogen levels in the liver and muscle tissues were altered by benzene while glucose in the blood remained largely unchanged.

Key words: benzene, glycogen, liver, muscle, blood.

INTRODUCTION

We are exposed to thousands of naturally occurring and synthetic chemicals over our lifetime. Many chemicals are essential for life and are beneficial, while exposure to other chemicals can be harmful and affect our health (BCERF 2002). Benzene enters into the environment through of both natural and human activities, which was first discovered in 1985 by Michael Faraday (Luoping *et al*., 2002). Benzene is a ubiquitous, highly flammable, colorless liquid that is a known hematotoxin, myelotoxin and human leukemogen (Boca *et al*., 2001). Some industries use benzene to create other chemicals that are used to make plastics, resins, nylons and synthetic fibers. In addition, benzene is used to make some types of rubbers, lubricants, dyes, detergents, drugs and pesticides. People working in industries that make or use benzene may be exposed to high levels (ASTDR 1997). Examples of occupational exposure include plumbers of the gas distribution network, laboratory technicians, sheet-metal workers and mechanics in thermal or hydroelectric production plants. Intensive exposure to benzene, as a solvent, when cleaning some materials and as gasoline for motor vehicles, etc, increases its effects (Pascal *et al*., 2002). Although only a relatively small number of individuals are occupationally exposed to benzene, the general population is exposed in gasoline, automobile exhaust and diesel fuel. Furthermore, benzene is

present in cigarette smoke and smoking is the main source of benzene exposure for many people (Luoping *et al*. 2002).

According to Mark and Gary (1999), vehicle exhausts and industrial emissions account for ~20% of human exposure, while exposure to cigarette smoke accounts for ~50% (Mark and Gary 1999). Estimates indicate that occupational exposure affects as many as 238000 people (ASTDR 1997). Benzene, being lipid soluble, is transported in the blood and absorbed by the red cell membranes. It tends to accumulate in tissues with a high lipid content and about 50% of the absorbed dose may be eliminated unchanged in the exhaled air, while the remainder is metabolized in the liver, primarily by cytochrome P-450 2E1 systems (Hannumantharao *et al*., 2001).

During the metabolism of benzene several intermediate products are produced. The first step in benzene metabolism is the formation of an epoxide or benzene oxide catalyzed by cytochrome P-450 2E1, followed by several alternative metabolic pathways (U.S. EPA 2002). The major metabolites of benzene, which is largely metabolized in the liver, are phenol, hydroquinone and catechol. These and other intermediate products reach target tissues by the hepatic portal vein and may cause much direct and indirect damage (U.S. EPA 2002). Metabolism is necessary for the expression of the characteristic hematotoxic and carcinogenic effects of benzene. Despite extensive research, no single metabolite has been identified as responsible for all the toxic effects of benzene and the weight of evidence points to an interaction of several metabolites (U.S. EPA 2002). In humans, exposure to high concentrations of benzene can result in central nervous system depression, cardiac arrhythmia, respiratory failure and death. Low concentrations may lead to headaches, dizziness, weakness, nausea, vomiting etc (WHO 1993). Chronic exposure to high doses of benzene produces characteristic bone marrow toxicity expressed as anemia, leukocytopenia, lymphocytopenia, thrombocytopenia and myelodyplastic syndromes (William *et al*., 2002).

Ronda *et al*. (2001), in an *in vitro* assay, demonstrated that most benzene metabolites are capable of inhibiting topoisomerase II, which has been shown to induce leukemia in humans. Inhibition of this enzyme by benzene metabolites may also play a role in the carcinogenic effects of benzene (Frantz and Chen, 1996). In recent years several *in vitro* studies have been conducted, which demonstrate the ability of benzene and/or various metabolites to induce DNA damage (Luoping *et al*., 1999; Albin *et al*., 2000; Cynthia *et al*., 2001; Mehmet *et al*., 2002; Luoping *et al*., 2002; William *et al*., 2002). Organisms have developed defence mechanisms against toxic molecules such as benzene. NQO1; quinone oxidoreductase 1 is an enzyme with the ability to detoxify a number of natural and synthetic compounds and to activate certain anticancer agents. It plays an important role in cancer chemoprevention. NQO1 was observed to have protective effects against benzene toxicity and various cancers, including leukemia (Martyn, 1999).

Modifications in the activities of catalase, superoxide dismutase and glutathione S-transferase in the liver, kidneys and serum were recorded in rats and rabbits (Serif *et al*., 1999). Benzene and its metabolites can attach to hepatic

enzymes, proteins, monosaccharides and disaccharides leading to changes in the level of glucose in the blood and in energy metabolism. Benzene can have carcinogenic and non-carcinogenic effects in all organisms. The occurrence of glycogen and benzene metabolism in the same organ has led to the consideration of a probable link between benzene and glycogen.

Our study investigates the effects of benzene administration on glycogen levels in the liver and muscle tissues and blood glucose in relation to sex and time.

MATERIAL AND METHODS

Animals

Swiss albino rats (*Rat rattus norvegicus*) between 200-250 g body weight were acquired from the Guinea Pig Feeding and Research Centre of Uludag University Medical Science Faculty. Four rats were assigned to the control group for each trial period, while eight rats were used from the benzene-treated group for each trial period. Thus, a total of 178 animals were used, out of which 168 were evaluated, 10 animals having died during the experiments.

Chemicals

Benzene (Cyclohexatriene) 99.5%, trichloracetic acid pure, potassium chloride, heparin, ethyl alcohol 99.5%, sulphuric acid 99.5%, anthrone, and thiourea were obtained from Merck.

Analysis of glycogen level in tissues

The experimental animals were left without food and water for 24 hours, and then 100 mg kg^{-1} dose of benzene (99.5%) was injected intraperitoneally using sterile injectors of 1 ml. Following the injection, food and water were regularly given to the animals in both the control and benzene treated groups until the trial periods were completed. The rats were decapitated by cervical dislocation 0, 2, 4, 8, 16, 32 and 64 hours after the injection. The tissues of liver and muscle were quickly removed and perfused in 10% trichloracetic acid. Both tissues were stored at -17 ^{o}C. Tissues were homogenized in trichloracetic acid (1/3 mass/volume) at 15000 rpm for 5 min for liver and at 21000 rpm for 15 min for muscle in a T-line laboratory stirrer tissue homogenizer. Glycogens were precipitated according to the method of Joseph *et al*. (1961), and designated according to Nicholas *et al*. (1955). In this study, Cecil 2000 spectrophotometry was used.

Determination of glucose level in the blood

Blood was taken from the rat hearts in heparinised syringes. The blood was centrifuged at 10000 rpm for 10 min in a Kubata 5800 type centrifuge. Glucose was determined using a Technican RA-1000 autoanalyser.

Statistical analysis

Statistical analysis of the results included multiple comparisons with ANOVA (LSD test) for paired and unpaired data to determine the differences within and between the control and benzene-treated groups. The independent t test was used to determine the statistical significance of differences according to sex in both groups. Data are expressed as mean±SE, with $P<0.05$ being considered significant. All analyses were performed with SPSS software.

RESULTS

Glycogen level in the liver

The effects of benzene on glycogen levels in the liver of male and female rats are given in Figure 1. The statistical data may be seen in Table 1 and 2.

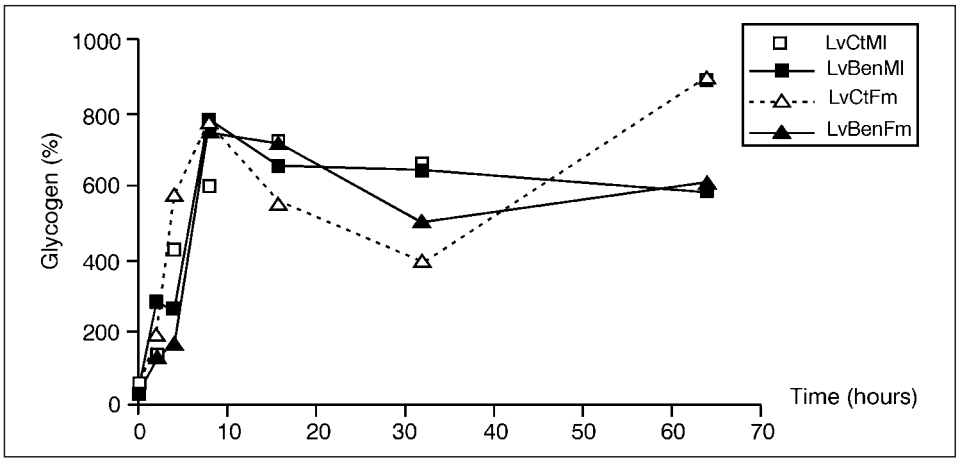

Figure 1. Changes in glycogen levels in liver tissue of control and benzene-treated rats with respect to time and sex
LvCtMl : Liver Control Male, LvBenMl : Liver Benzene Male,
LvCtFm : Liver Control Female, LvBenFm : Liver Benzene Female

When the glycogen levels were evaluated according to sex and time, similar changes were observed in both groups. Immediately after the injections, the mean glycogen level in the liver of the benzene-treated group of male rats was half that of the control group. This difference was statistically significant ($p<0.05$). Two hours later the glycogen level of the control group had increased markedlly to reach the maximum level at the end of the experiment (64 hours). Maximum glycogen level was reached in the benzene-treated group at the eighth hour (777±22.2 mg/100 g), at which time the value was not significantly different from

Table 1. Changes in glycogen levels in the liver tissue of control and benzene-treated rats with respect to time and sex

Time Hours $_r$		0 Mean±SE	2 Mean±SE	4 Mean±SE	8 Mean±SE	16 Mean±SE	32 Mean±SE	64 Mean±SE
Male	Control	59.23±8.42 ax	133.24±22.80 ax	423.52±10.94 bx	599.15±41.13 cx	721.29±9.96 dx	658.75±81.99cdx	885.96±35.84ex
	Benzene	28.33±4.70 ay	282.41±86.29 ax	264.17±12.46 ay	777.37±221.52 bx	661.87±104.09 bx	640.80±79.14 bx	580.91±22.98by
Female	Control	33.82±2.33 ax	195.15±18.35 ax	576.73±52.02 bx	777.66±164.0 cx	552.98±12.84 bx	393.30±20.0 bx	890.92±51.42cx
	Benzene	38.59±4.15 ax	129.78±15.25aby	169.48±31.66 by	749.05±67.25 cx	715.67±50.75 cdy	449.19±48.18 ex	602.20±47.24dey

Table 2. Changes in glycogen levels in the liver tissue of control and benzene-treated rats according to sex within each group

Time Hours $_r$		0 Mean±SE	2 Mean±SE	4 Mean±SE	8 Mean±SE	16 Mean±SE	32 Mean±SE	64 Mean±SE
Control	Male	59.23±8.42 ax	133.24±22,80 ax	423.52±10.94 bx	599.15±41.13 cx	721.29±9.96 dx	658.75±81.99cdx	885.96±35.84 ex
	Female	33.82±2.33 ay	195.15±18.35 ax	576.73±52.02 by	777.66±164.0 cx	552.98±12.84 by	393.30±20.0 by	890.92±51.42 cx
Benzene	Male	28.33±4.70 ax	282.41±86.29 ax	264.17±12.46 ax	777.37±221.52bx	661.87±104.09bx	640.80±79.14 bx	580.91±22.98 bx
	Female	38.59±4.15 ax	129.78±15.25 abx	169.48±31.66 by	749.05±67.25 cx	715.67±50.75cdx	449.19±48.18 ex	602.20±47.24dex

* Data shown with the same symbols in the horizontal column are not different from each other at 0.05 probability (abcd).
** Data shown with the same symbols in the vertical column are not different from each other at 0.05 probability (xy).
r : All data in the table showed (mg /100g) glycogen levels.
SE : Standard Error.

the control. Glycogen levels were significantly lower in the benzene treated group at other times (1.6 times at 4 hours and 1.5 times at 64 hours) ($p<0.05$) (Table 1).

When the levels of glycogen in the liver of female rats were investigated, an increase was observed from 0 hours to 8 hours in both groups. Then they decreased from 8 hours to 32 hours in both groups followed by an increase at 64 hours. However, the glycogen levels of the liver in benzene-treated groups at 2, 4 and 64 hours ($p<0.05$), were significantly lower than those in the control group but 1.3 times higher at 16 hours ($p<0.05$) (Table 1).

The effects of benzene on glycogen levels in the liver according to sex are given in Table 2. Statistically significant differences were found between control groups at 0, 4, 16 and 32 hours, ($p<0.05$) the values being lower in the females except at 4 hours. This was the only time there was a statistically significant difference between male and female benzene-treated groups ($p<0.05$).

Glycogen level in muscle

The effects of benzene on glycogen levels in the muscle are given in Figure 2. The statistical data may be seen in Tables 3 and 4.

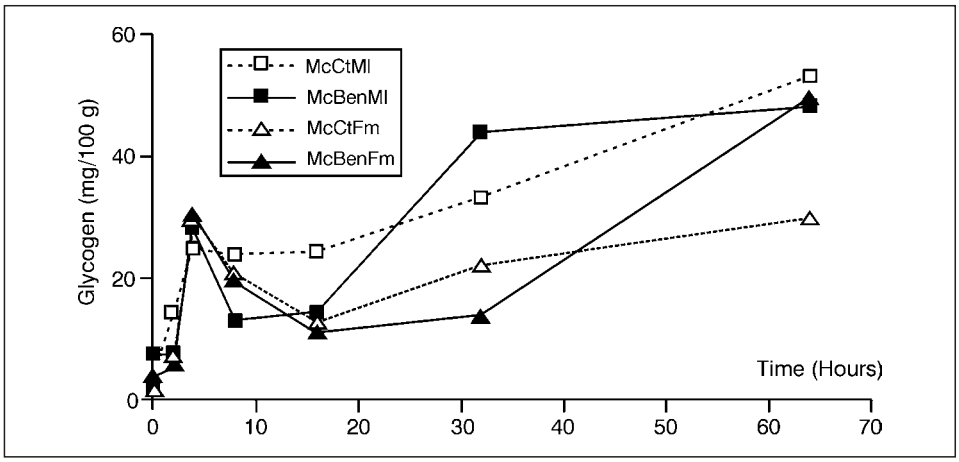

Figure 2. Changes in glycogen levels in muscle tissue control and benzene-treated rats with respect to time and sex
McCtMl : Muscle Control Male, McBenMl : Muscle Benzene Male,
McCtFm : Muscle Control Female, McBenFm : Muscle Benzene Female

The profiles glycogen levels in muscle in the control and benzene-treated groups were similar in males and females. Immediately after treatment, the glycogen level in the benzene-treated male group was three times as much as in the control group of male rats. An increase in glycogen level until the 4^{th} hour was observed in the benzene-treated group. Although a decrease occurred at 8 hours,

Table 3. Changes in glycogen levels in the muscle tissue of control and benzene-tbolites are cath respect to time and sex

Time Hours $_r$		0 Mean±SE	2 Mean±SE	4 Mean±SE	8 Mean±SE	16 Mean±SE	32 Mean±SE	64 Mean±SE
Male	Control	2.56 ± 0.29 ax	14.31 ± 0.41 bx	24.89 ±2.23 bcx	23.75 ± 2.90 bcx	24.19±3.64 bcx	33.04 ± 1.98 cx	52.63 ± 8.78 dx
	Benzene	7.52 ± 0.58 ay	7.46 ± 0.57 ay	28.19 ± 2.51 bx	12.86 ± 2.58 acy	14.28 ± 149 cy	43.75 ± 2.09 dy	48.10 ± 3.13 dx
Female	Control	2.63 ± 7.22 ax	6.71 ± 0.69 abx	29.79 ± 3.34 cx	20.65 ± 1.6 dx	12.48 ± 0.99 bx	21.86 ± 4.63 dx	29.36 ± 2.73 cx
	Benzene	3.87 ± 0.37 ay	5.04 ± 1.24 ax	30.85 ±10.53 bx	19.29 ± 1.46 bcx	10.86± 1.72 acx	13.65 ± 0.61 acx	48.76 ± 1.39 dy

Table 4. Changes in glycogen levels in the muscle tissue of control and benzene-treated rats according to sex within each group

Time Hours $_r$		0 Mean±SE	2 Mean±SE	4 Mean±SE	8 Mean±SE	16 Mean±SE	32 Mean±SE	64 Mean±SE
Control	Male	2.56 ± 0.29 ax	14.31 ± 0,41 bx	24.89 ±2.23 bcx	23.75 ± 2.90bcx	24.19 ±3.64 bcx	33.04 ± 1.98 cx	52.63 ± 8.78 dx
	Female	2.63 ± 7.22 ax	6.71 ± 0.69 aby	29.79 ± 3.34 cx	20.65 ± 1.6 dx	12.48 ± 0.99 by	21.86 ± 4.63 dx	29.36 ± 2.73 cy
Benzene	Male	7.52 ± 0.58 ax	7.46 ± 0.57 ax	28.19 ± 2.51 bx	12.86 ±2.58 acx	14.28 ± 149 cx	43.75 ± 2.09 dx	48.10 ± 3.13 dx
	Female	3.87 ± 0.37 ay	5.04 ± 1.24 ax	30.85 ±10.53 bx	19.29 ±1.46 bcx	10.86 ± 1.72 acx	13.65 ± 0.61 acy	48.76 ± 1.39 dx

* Data shown with the same symbols in the horizontal column are not different from each other at 0.05 probability (abcd).
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r : All data in the table showed (mg /100g) glycogen levels .
SE : Standard Error

following this time the glycogen levels increased until the end of the experiment. At 2, 8 and 16 hours values were significantly lower and at 0 and 32 hours they were significantly higher than in the male control group ($P<0.05$).

An increase was observed in glycogen level in both groups of female rats until the 4th hour. Later, a decrease occurred until the 16th hour followed by an increase at 32 and 64 hours. A statistically significant difference was seen only at 0 and 64 hours when the glycogen levels of the female control and benzene groups were compared ($P<0.05$, Table 3).

The effects of benzene on glycogen levels in muscle of the groups of rats with respect to sex are given in Table 4. A statistically significant difference between the sexes was only seen in the control group at 2, 16, and 64 hours ($P<0.05$). The difference between male and female rats in the benzene-treated groups at 0 and 32 hours was also statistically significant ($P<0.05$, Table 4).

Level of glucose in the blood

The effects of benzene on levels of glucose in the blood of male rats are given in Figure 3 and of female rats in Figure 4. The profiles were similar for all groups. The statistical data may be seen in Table 5.

Levels of glucose in the blood were 15.7% lower in the experimental group of male rats compared with the controls at the 32nd hour. This difference was statistically significant ($P<0.05$, Table 5). While the maximum glucose level in the control group was observed at 32 hours, it was determined in the benzene-treated group at 4 and 64 hours.

A significant difference between control and benzene groups was observed at 2 and 64 hours when the level of glucose was compared for female rats ($P<0.05$). The maximum glucose level in both female control and benzene-treated groups occurred at 32 hours.

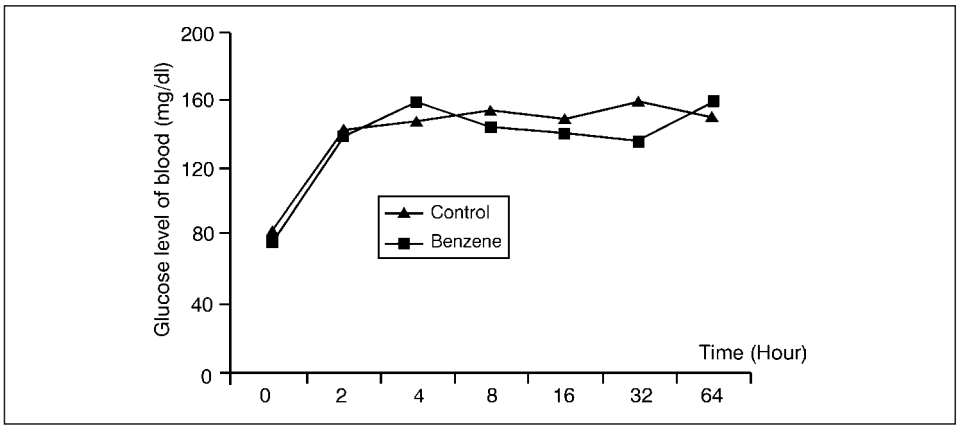

Figure 3. Changes in levels of glucose in the blood of control and benzene-treated groups of male rats with respect to time

Table 5. Changes in blood glucose levels of control and benzene-treated rats with respect to time and sex

Time Hours $_r$		0 Mean±SE	2 Mean±SE	4 Mean±SE	8 Mean±SE	16 Mean±SE	32 Mean±SE	64 Mean±SE
Male	Control	79.75±3.09 ax	143.75±3.22abx	149.25±4.60acx	155.0±7.4 bcdx	149.75±5.40adex	159.5±6.11 cex	150.25±4.62adex
	Benzene	72.75±4.38 ax	139.5±4.36 ax	159.5±7.19 bx	145.25±6.61abx	139.5±3.79 ax	134.5±3.86 ay	159.5±7.30 bx
Female	Control	75.25±4.02 ax	140.25±3.30abx	142±1.47 abx	150.25±6.45bcdx	144.5± 3.86 acx	157.25±5.85dex	148±4.69 bcex
	Benzene	65.25±6.96 ax	129±1.82 ay	139±5.44 abx	133.25±6.19abx	130.25±9.91abx	150.25±11.54bx	124.25±6.78 ay

Table 6. Changes in blood glucose levels of control and benzene-treated rats according to sex

Time Hours $_r$		0 Mean±SE	2 Mean±SE	4 Mean±SE	8 Mean±SE	16 Mean±SE	32 Mean±SE	64 Mean±SE
Control	Male	79.75±3.09 ax	143.75±3.22 abx	149.25±4.60 acx	155.0±7.42 bcdx	149.75±5.40adex	159.5±6.11 cex	150.25± 4.62adx
	Female	75.25±4.02 ax	140.25±3.30 abx	142±1.47 abx	150.25±6.45bcdx	144.5±3.86 acx	157.25±5.85 dex	148±4.69 bcex
Benzene	Male	72.75±4.38 ax	139.5±4.36 ax	159.5±7.19 bx	145.25±6.61 abx	139.5±3.79 ax	134.5±3.86 ax	159.5±7.30 bx
	Female	65.25±6.96 ax	129±1.82 ax	139±5.44 abx	133.25±6.19 abx	130.25±9.91 abx	150.25±11.54 bx	124.25±6.78 ay

* Data shown with the same symbols in the horizontal column are not different from each other at 0.05 probability (abcd).
** Data shown with the same symbols in the vertical column are not different from each other at 0.05 probability degildir (xy).
r : All data in the table showed (mg/100g) glycogen levels.
SE : Standard Error

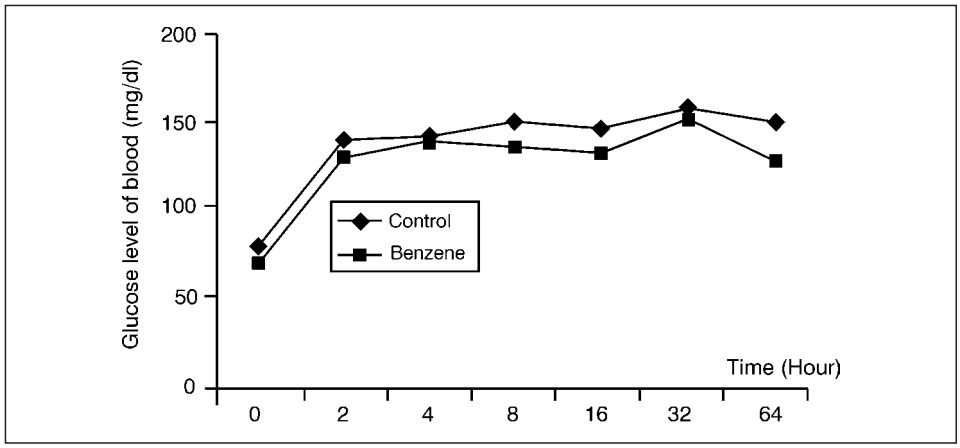

Figure 4. Changes in levels of glucose in the blood of physiological serum as control and benzene-treated groups of female rats with respect to time

The effects of benzene on levels of glucose in the blood dependent upon sex are given in Table 6. No statistically significant difference was found when the levels of glucose in the blood in the control groups of male and female rats were compared ($P>0.05$). The difference seen between male and female rats in the benzene-treated group at 64 hours was statistically significant ($P<0.05$).

DISCUSSION

In this study, an increase in glycogen level in the liver was observed in both control and the benzene-treated groups at the 2nd hour after the treatment. The reason for this increase is that the animals, which had been fasting for 24 hours, were given nourishment after the injection. Because these increases occurred in both groups, they could be counted as metabolic increases.

Nevertheless, the glycogen level of the benzene-treated groups was found to be lower than that of the control groups in both liver and muscle tissues. This probably resulted from the effect of benzene or its metabolites on glycogen metabolism. Benzene is rapidly metabolized in the liver and may cause toxic effects. The metabolism of benzene in the liver will also affect the synthesis and catabolism of glycogen, which occurs in the same organ and may be the reason for the decrease in glycogen level. Another cause of this decrease might have been differences in nourishment after the injection, because the benzene-treated group may have consumed less feed after the injection.

After the second hour, the liver glycogen levels increased in the control and the benzene-treated groups to reach maximum levels at the 8th hour (Table 1). Although these increases were metabolic, the mean glycogen level in the benzene-treated group was lower than that of the control group. The reason for

the occurrence of lower glycogen levels in the benzene-treated group may be the effect of some metabolites of benzene, such as phenol and benzene oxide on glycogen metabolism. Benzene is rapidly metabolized in the liver, producing primary benzene oxide and various phenolic products. These metabolites may cause toxic effects in the liver and other tissues.

Andrew *et al*. (1999) investigated benzene metabolism and benzene oxide distribution in rat bone marrow, liver, kidneys and zymbal glands. Benzene oxide or phenol was detected in the liver and kidneys but not in the zymbal glands and bone marrow. Therefore, it was proposed that benzene oxide is rapidly formed, and is carried by blood circulation to other tissues, where it causes toxicity (Andrew *et al*., 1999). These findings seem to support our idea. In addition, benzene produces various intermediate products such as hydroquinone, catechol and trihydroxybenzene. In recent studies, it was concluded that intermediate products that appear because of benzene metabolism, and the relationship which exists between them, produce greater toxicity than benzene itself (Richard *et al*., 2003).

Giorgio *et al*. (2001) analysed trans-muconic acid levels in urine samples after low exposure (0.1-2 ppm, 6 h) of rats by inhalation. Trans-trans muconic acid level was detected at 50 ng ml^{-1} in rat urine in less than 20 minutes. It is therefore clear that benzene metabolites arise in a short time and may affect glycogen metabolism. It was observed that the liver glycogen levels in the benzene-treated and control groups tended to decrease after the 8th hour.

Benzene, an aromatic hydrocarbon, may cause a decrease in glycogen level by affecting the enzymes or enzyme systems that play a role in glycogen metabolism. Soman *et al*. (1974) found that glycogen phosphorylase *b* of rabbit muscle was inhibited by soluble aromatic compounds. These chemical variations also affect the activation of enzymes. Glycogen phosphorylase occurs in two forms: the active form phosphorylase *a* and the relatively inactive form phosphorylase *b*. Phosphorylase *b* can in turn be transformed back into active phosphorylase *a*-by another enzyme, phosphorylase kinase. If this system suffers damage, phosphorylase *a* always activates the breakdown of glycogen in skeletal muscle and the liver. Therefore, the decreases in glycogen seen in liver and muscle tissues may be caused by the effect of benzene or its metabolites on enzyme mechanisms in this pathway.

In an other study, Kaminski *et al*. (1985) investigated the activities of $NADH_{2,}$ glucose-6 phosphatase and ATPase, administering benzene to mice for 24 hours intraperitoneally. A decrease in the amount of glycogen in hepatocytes, a deviation in the amount of neutral lipids and a decrease in enzyme activities of mitochondria were noted. Glucose-6 phosphate was converted to glucose by glucose-6 phosphatase. As a result of a decrease in glucose-6 phosphatase activity caused by the effect of benzene, there may be irregularities in the level of liver and blood glycogen. Therefore, a decrease in the glucose level of blood may be seen, a liver glycogen is a main source of glucose in the blood. In a different study, some variations were determined in glucose-6 phosphate and glucose dehydrogenase activities in rats treated with benzene. A decrease in glucose-6 phosphatase activity and are increase in glucose dehydrogenase activities in rats

were found (Andrey and Vladimir 1998). Fatma and Egemen (2003) investigated the effects of benzene on the liver glutathione S-transferase (GST) enzyme *in vitro*. An inverse correlation between GST conjugation activity and concentration of benzene, as well as reaction time and variations in the Vmax and Km value for CDNB (1-chloro-2,4-dinitrobenzene) of the enzyme were observed. These findings seem to support our opinions.

Another reason for the decrease of glycogen level in the liver may be genetic damage by benzene. It was shown that benzene and its metabolites may cause genetic damage (oxidative DNA damage, numerical and structural chromosomal damage, micronuclei etc) *in vitro* and *in vivo* by Richard *et al*. (2003).

The glycogen level in the liver of control and benzene-treated groups rapidly increased after 32 hours. However, this increase in the benzene-treated group was lower than the control group. This led us to believe that benzene or its metabolites were being gradually expelled from the body and that their effects were gradually reduced. It was found that more than 95% is discarded in the urine and about 3% by respiration within 48 hours in studies of benzene metabolism with ^{14}C in low doses in rats. As for high doses, it was shown that excretion levels in faeces were low and discard by respiration increased from 9% to 50% (U.S. EPA 2002 and Qingshan *et al*., 2002). In one respect, these studies seem to support our findings.

Significant decreases were found in the level of glycogen in muscle after 4 hours. We consider that the first products formed by benzene metabolism in the liver may reach muscle tissue by circulation and show their effects there. An increase in glycogen level of muscle tissue occurred after 16 hours. This increase may have been caused by removal of the molecules from their surroundings by detoxification. The levels of glycogen in muscle were lower than the level of glycogen in the liver. However, glycogen in muscle is important for metabolism.

An increase was seen in the level of blood glucose beginning immediately after treatment. We thought that this increase was a metabolic increase. However, it was found that the level of blood glucose in the benzene-treated group was lower in relation to the control group at certain times. The cause of the decrease may be metabolism of benzene. Brugnone *et al*. (1999) investigated the occupational effect of benzene on 150 workers employed in petrol stations and refineries. All workers provided blood samples after the end of work and on the following morning before resuming work. Overall, median blood benzene of all workers was 251 ng/l at the end of the work and 174 ng/l the following morning. The benzene concentrations measured in blood collected the following morning proved to be significantly lower than those measured at the end of the shift (Brugnone *et al*. 1999). This study supports our opinion that the effects of benzene on glycogen levels depends on time.

After an increase in the level of blood glucose up to 4 hours, levels decreased below control values in the benzene treated rats, while an increase in glycogen level of the liver until 8 hours was seen. We thought that benzene and its metabolites reached the blood before the other tissues and showed its effects there earlier. Benzene is first metabolized in the liver and afterwards may be carried to other tissues by circulation of the blood. For this reason, benzene

showed its effects in blood more rapidly than in the other tissues. Yeowell *et al*. (1998) investigated the effect on hemoglobin and albumin of benzene-oxide occurring in workers exposed to high doses of benzene. They observed that benzene oxide reacts with cysteinyl residues in hemoglobin and albumin to form protein adducts, which are presumed to be specific biomarkers of exposure to benzene. In this way, the fact that benzene reacts with different molecules may be the reason for showing a toxic effect and for changes in glucose levels. Benzene, by affecting cells and enzymes in blood, may cause a toxic effect. Moszczynski *et al*. (1978) investigated the effect of benzene, toluene, and xylene on peripheral blood neutrophils in 106 workers. An increase occurred in acid hydrolase activity in neutrophil while a decrease was found in glycogen levels. At the end of the experimental period, enzyme activities decreased, while aggregations were detected in glycogen and lipids in neutrophils. It was shown that these alterations in neutrophil were caused by the toxic effect of benzene. These discussions seem to support our finding that benzene may cause a decrease in blood glucose. Another study by Micu *et al*. (1972) showed that leukocyte alkaline phosphatase, endolymphocytic glycogen and lymphocytic nuclear RNA amounts exhibited abnormal values after occupational exposure to benzene. It was shown that benzene and its metabolites also blocked glucose permeating through plasma membranes by Schaw *et al*. (1981).

The glycogen levels of muscle and liver of female rats were much lower than those of male rats. Glucose levels of blood showed the same trends as glycogen levels of muscle and liver. In the in-vitro study by Miriam *et al*. (1997), it was shown that erythrocytes of adult female rats were much more resistant to the toxic effect of benzene metabolites.

Carbohydrate metabolism is important for metabolic energy. It is clear that the damage by the effects of benzene will probebly affect all systems. In a study by Egemen *et al*. (2003), it was shown that estradiol and testosterone activities in the serum, lactate dehydrogenase, alkaline phosphatase, alanine amino transferase, aspartate amino transferase, and pyruvate kinase activities in the liver and kidney of rats given a 100 $mg.kg^{-1}$ dose of benzene were affected. Consequently, they found that benzene caused some changes (increases then decreases) in enzymes and hormone activities in different tissues of rats.

Thus, it was determined that benzene causes a decrease in glycogen levels of muscle and liver. At the same time it also affects the glucose level of blood. We consider that determining activations and inhibitions of enzymes in glycogen metabolism would show as a definite effect of toxic metabolites on these systems.

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REFERENCES

1. *Albin M, Björk J, Welinder H, Tinnerberg H, Mauritzson N, Johansson B, Billström et al., 2000. Acute* myeloid leukemia and clonal chromosome aberration in relation to past exposure to organic solvents, *Scand J Work Environ Health,* 26, 482-91.
2. *Andrew BL, Karen YC, Suramya W, Thomas AM, Stephen MR*, 1999. Investigation of benzene oxide in bone marrow and other tissues of F344 rats following metabolism of benzene *in vitro* and *in vivo*. *Chemico-Biological Interac*, 122, 41-58.
3. *Andrey LS, Vladimir IP,* 1998. Age-dependent changes in rat liver microsomal membrane structure and functions under benzene treatment, *Mech Ageing Develop,* 106, 273-82.
4. ATSDR: Agency for Toxic Substances and Disease Registry, 1997. Toxicological Profile for Benzene (Update). U.S. Department of Health and Human Services, Atlanta, GA.
5. BCERF: Breast Cancer and Environmental Risk Factors. 2002. Cornell University Program in New York State, Fact Sheet 45.
6. *Boca R, Mark RL, Cammey EC, Paul MS,* 2001. A review of quantitative studies of metabolism, *Crit Rev Toxicol*, 31, 3, 285.
7. *Brugnone F, Perbellini L, Romeo L, Cerpelloni M, Bianchin M, Tonello A,* 1999. Benzene in blood as a biomarker of low level occupational exposure, *Sci Tot Environ,* 235, 247-52.
8. *Carrol NV, Longley RW, Roe JH,* 1956, The Determination of Glycogen in Liver and Muscle by Use of Anthrone Reagent, *J. Biol Chem*. June, 220, 2, 583-93.
9. *Cynthia RG, Rosemary W, Dan HM, Maria GP,* 2001. Dermal benzene and trichloroethylene induce aneuploidy in immature hematopoietic subpopulations, *Environ Molec Mutagen,* 37: 185-94.
10. *Egemen D, Suriye G, Hüseyin A*, 2003, The effect of benzene on the activity of some enzymes and hormones in dýfferent týssues of rats. *Acta Veterinaria*, 53, 87-101.
11. *Fatma A, Egemen D*, 2003. The *in vitro* effect of benzene on liver glutathione S-transferase enzyme activity, *Fen Bilm Derg,* 28-03.
12. *Frantz CE, Chen H*, 1996, Inhibition of human topoisomerase II *in vitro* by bioactive benzene metabolites, *Environl Health Persp Suppl,*104, 6, 1319, 5p, 4 charts.
13. *Giorgio M, Elbert AH, Teresa C, Luigi M*, 2001. Improved coupled column liquid chromatographic method for high-speed direct analysis of urinary trans, trans- muconic acid, as a biomarker of exposure to benzene, *J Chromat B,* 751, 331-9.
14. *Hannumantharao GR, Smita M, Vrinder SP, Ekta K, Yogesh KT, Vishwajeet R et al,,* 2001. Chemoprevention of benzene-induced bone marrow and pulmonary genotoxicity. *Teratogen, Carcinogen Mutagen,* 21, 181-7.
15. *Joseph H, Roe JM, Bailey R, Richart G, John N*, 1961. Complete removal of glycogen from tissues by extraction with cold trichloroacetic acid solution, *J Biol Chem,* 236, 5.
16. *Kaminski M, Jonek J, Kaminska O, Gruszeczka B, Koehler B*, 1985, Histochemical and histoenzymatic changes in mouse liver in subacute benzene intoxication, *Med Intern,* 23, 2,115-20.
17. *Luoping Z, David AE, Martyn TS,* 2002, The nature of chromosomal aberrations detected in humans exposed to benzene, *Crit Rev Toxicol,* 32, 1, 1-42.
18. *Luoping Z, Nathaniel R, Yunxia W, Richard BH, Songnian Y, Nina TH et al,* 1999. Benzene increases aneuploidy in the lymphocytes of exposed workers: A comparison of data obtained by fluorescense in situ hybridization in interphase and metaphase cell, *Environ Mol Mutagen, 34, 260-8.*
19. *Mark WP, Gary PC*, 1999, Species comparison of hepatic and pulmonary metabolism of benzene. *Toxicol*, 139, 207-17.
20. *Martyn TS*, 1999, Benzene, NQQ1 and genetic susceptibility to cance, *Proc Natl Acad Sci,* USA, 96, 7624-6.
21. *Mehmet T, Eyyüp R, Hasan BI, Ahmet K,* 2002, Chromosome aberration and sister chromatid exchange in workers of the iron and steel factory of Iskenderun, Turkey, Teratogen, *Carcinogen Mutagen,* 22, 411-23.

22. *Micu D, Mihaillescu E, Vilau C, Tarpa A, Chircu V, Zgoanta C*, 1972, The value of some cytoenzymochemical investigations of the leukocytes and platelets in estimating the effects of occupational exposure to benzene, vinyl chloride and carbon disuphide, *Eur J Pharm,* 20, 97-103
23. *Miriam C, Carroll AS,* 1997, Gender-and age- specific cytotoxic susceptibility to benzene metabolites *in vitro, Toxicol Sci, 41, 42-8.*
24. *Moszczynski P, Lisiewicz J,* 1978. Enzymes of neutrophils in workers occupationally exposed to benzene, toluene and xylene, *Acta Histochem*, 61,1,1-19.
25. *Pascal G, Ellen I, Anne C, Anne CC, Marcel G*, 2002, Leukemia in relation to occupational exposures to benzene and other agents: a case-control study nested in cohort of gas and electric utility workers, *Am J Ind Med*, 42, 87-97.
26. *Qingshan Q, Roy S, Guilan L, Ximej J, Lung CC, Beverly C et al*, 2002, Hematological changes among Chinese workers with a broad range of benzene exposures, *Am J Ind Med,* 42, 275-85.
27. *Richard A, Harvey C, Matthew WH, Eric M, Stephen O, Julian P et al, 2003,* The use of non- tumor data in cancer risk assessment: reflection on butadiene, vinyl chloride, benzene, *Regul Toxicol Pharmacol 37, 105-32.*
28. *Ronda KB, Ebba UK, David WP, Richard DI, David K*, 2001, Benzene metabolites antogonize etoposite-stabilized cleavable complexes of DNA topoisomerase II. *Blood,* 98, 3.
29. *Schraw PW, Regen MD, Harris MT*, 1981, Inhibition of glucose transport by benzoquinone and the addition product of benzoquinone and dithiothreitol. *Bioch Biophys Acta (BBA)-Biomembranes,* 649, 735-42.
30. *Soman G, Philip G*, 1974, Aromatic compounds as allosteric inhibitors of glykogen phosphorylase b, *Bioch BiophysActa (BBA)- Enzymology,* 358, 354-62.
31. *Serif A, Alper G, Murat O, Abdullah O, Kurtulus Y, Türker K et al, 1999,* The effect of benzene on serum, hepatic and renal glutathýone S transferase, superoxide dismutase, catalase of rat and rabbit, *Biochem Arch,* 15, 239-46.
32. U.S. EPA: U.S. Environmental Protection Agency. 2002. Toxicological Review of Benzene in Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-02/001F.
33. WHO: World Health Organization.1993. Environmental Health Criteria. 150, Benzene. Genova.
34. *William WA, Boris O, Carsten H*, 2002, Assessing DNA damage and health risk using biomarkers, *Mut Res,* 509, 153-63.
35. *Yeowell K, Rothman N, Smith MT, Hayes RB, Li G, Waidyanatha et al,* 1998, Hemoglobin and albumin adducts of benzene oxide among workers exposed to high levels of benzene. *Carcinogen*, 19, 9, 1567-71.

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Özdikicioglu Ferda *et al.* Effects of benzene on glycogen levels of liver and muscle tissues and on blood glucose of rats

EFEKTI BENZENA NA NIVO GLIKOGENA U JETRI I MIŠIĆNOM TKIVU I NA GLUKOZU U KRVI PACOVA

ÖZDIKICIOGLU FERDA and DERE E

SADRŽAJ

U ovoj studiji je švajcarskim albino pacovima intraperotinealno aplikovano 100 $mg.kg^{-1}$ benzena, koji se često koristi u industriji i poznat je kao toksičan i kancerogen agens. Effekti benzena su ispitivani na osnovu promena u koncentraciji glikogena u jetri i mišićnom tkivu, kao i na osnovu promena glikemije nakon 0, 2, 4, 8, 16, 32 i 64 h.

Utvrđeno je da povećanje koncentracije glikogena u jetri i mišićima zavisi od načina ishrane kako kod kontrolne grupe tako i kod pacova tretiranih benzenom. Toksični efekat benzena se gubi nakon 64 h. Varijacije u nivou glukoze ne pokazuju značajne razlike između polova, osim u pojedinim periodima.

Dobijeni rezultati ukazuju da benzen utiče na koncentraciju glikogena u jetri i mišićima ali ne i na glikemiju.