STUDIES ON POISONING BY BENZENE AND ITS HOMOLOGUES

(8) EFFECT OF ENVIRONMENTAL TEMPERATURE ON SUSCEPTIBILITY IN BENZENE POISONING

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Individual differences in susceptibility to benzene have been observed. Systematic observations, however, using one kind of animal and one method have not been previously reported.

Accordingly, the individual differences in susceptibility to benzene from the viewpoint of environment will be analyzed in the present paper.

First of all, the effect of ambient temperatures on susceptibility in chronic benzene poisoning was examined.

Thirty male seven-week-old Donryu rats weighing 130 g and bred at 21°C were acclimatized to 32°C, 21°C and 10°C (relative humidity 55 %) for eight days. The rats were then given subcutaneous injections of 1.0 g/kg of benzene or same dose of olive oil for twelve successive days. Every four days blood taken from the tail was examined.

The body weight of the benzene injected rats showed a decrease at all temperatures. Significant leucopenia was observed at 32°C and 21°C, while no change of leucocyte count occurred at 10°C.

From the above results, the rats exposed to a cold temperature were felt to be minimally intoxicated by benzene as compared with the rats exposed to the moderate or high temperatures.

Individual differences in susceptibility to benzene have been observed by many workers. Females are more susceptible to benzene than males^{1~4)}, and young animals more susceptible than adult^{4~5)}. Dogs on a low protein diet are much more susceptible to benzene than those on an adequate protein intake⁶⁾. Men who easily suffer from summer heat are easily

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susceptible to benzene⁷⁾. Furthermore, a susceptibility in chronic benzene poisoning can apparently be correlated with infection acquired prior to exposure to benzene^{8~10)}.

As to the environment, benzene poisoned rabbits at 1,000 m above sea level recovered easily from anemia and leucopenia than rabbits at sea level¹¹⁾. Moreover, workmen exposed to benzene vapours have been reported to show severe signs of intoxication in summer than in winter^{12~13)}. And, after the exposure to benzene rabbits in a high temperature (40°C) showed prolonged chronaxia compared with rabbits in a low temperature (20°C)^{12~13)}.

As for the effect of temperature on the susceptibility to chemicals, many papers have reported. In the experiment of inhalation of carbon monoxide, mice at a high temperature lay down and die easily comparing with mice in a cold temperature¹⁴⁾. Rabbits have been reported to be easily intoxicated to phosphate in a high temperature¹⁵⁾. Rabbits at 30°C were reported to show higher susceptibility to lead than those at 20°C¹⁶⁾, while rabbits at 0°C were more easily intoxicated by lead than those at 31°C¹⁷⁾. In an experiment carried out at 36°C, 26°C and 8°C to determine the lethal dose of 58 kinds of chemicals in rats, the acute toxicity at 36°C was more severe than the other two. However, exceptional cases were seen in strychnine, promazine and chlorpromazine¹⁸⁾.

The present experiment will show that rats bred in a high temperature are more susceptible to benzene than rats in a cold temperature.

MATERIALS AND METHODS

A four-way layout was used in the present experiment. Factors examined were benzene administration (B), temperature (T), experimental period (D) and experimental animals (R). As experimental animals, seven-week-old male rats of Donryu strain weighing 130 grams were used. They were fed with commercial pelleted food and water *ad libitum*.

Thirty rats were divided into six equal groups. The animals were divided on the basis of body weight, each group showing the same average and the same variation on the first day of the experiment. The animals of each group were kept at the desired temperature for eight days for acclimatization, and the experiment was performed for the successive twelve days. The ambient temperatures examined in the experiment were 32°C, 21°C and 10°C, with relative humidity 55 ± 5 %. Fifty percent benzene in olive oil (1.0 g/kg) were administered to the experimental animals of one group in each ambient temperature subcutaneously on the back every morning, and another group served as control was given the same dose of olive oil on the back subcutaneously as well.

The criteria used to evaluate the degree of benzene intoxication were appearance, body weight, hemoglobin content, erythrocyte count, leucocyte count and thrombocyte count of the blood taken from the tail. Erythrocyte and leucocyte counts were determined by the tube method¹⁹⁾, thrombocyte count by the modification²⁰⁾ of the Björkman method²¹⁾, and hemoglobin content by the cyanmethemoglobin method²²⁾. Every four days blood was taken from the rats in random order using table of random digits.

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Table 1. Effect of environmental temperature on susceptibility in benzene poisoning.

Source of variation	Body weight	Hemo-globin	Erythro-cyte	Leuco-cyte	Thrombo-cyte
Benzene administration (B)	**			**	
Temperature (T)				*	
Date (D)	**	**	**		**
B × T				**	
B × D	**	**		**	
T × D		**	*	*	
B × T × D					

Level of significance : * 5 % ; ** 1 %

Fig. 1. Effect of environmental temperature on body weight of benzene-administered rats.

(Upper : Hot temperature (32°C)
 Median : Moderate temperature (21°C)
 Lower : Cold temperature (10°C)
 (Solid line : Benzene group
 Divided line : Control group)

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RESULTS

1. *Body Weight*

The control animals showed a gain of body weight throughout the experiment. Administration of benzene, however, disturbed the growth of the animals. The interaction of benzene administration and experimental period was significant at 1 % level of probability, that is, benzene injected rats showed no increase of body weight with time.

2. *Erythrocyte*

The erythrocyte count showed a sudden decrease in the second period. The erythrocyte count of rats in a moderate temperature showed a greater and more rapid increase than that of rats in the other temperatures.

Fig. 2. Effect of environmental temperature on erythrocyte count of benzene-administered rats.

Fig. 3. Effect of environmental temperature on leucocyte count of benzene-administered rats.

3. *Leucocyte*

Leucopenia was observed in rats injected benzene, and benzene caused a progressive decrease in the leucocytes of the experimental animals. However, the control rats in a moderate temperature showed a gradual increase of leucocytes compared with the rats in the other temperatures. The benzene injected rats in a low temperature showed almost no decrease in leucocyte count.

Fig. 4. Effect of environmental temperature on thrombocyte count of benzene-administered rats.

4. Thrombocyte

In a middle period of the experiment, slight thrombocytosis was observed.

5. Hemoglobin

All animals showed a gradual increase of hemoglobin content throughout the experiment. The hemoglobin content of benzene administered rats became higher than that of the control rats with time, and the hemoglobin content of the control rats varied with temperature — the rats at a low temperature showed the lowest hemoglobin content and the rats at a high temperature the highest at the end of the experiment.

Fig. 5. Effect of environmental temperature on hemoglobin content of benzene-administered rats.

DISCUSSION

It has been reported that workmen exposed to benzene vapours show severe signs of intoxication in summer than in winter¹²⁻¹³). Chronaxy measurement on rabbits showed that benzene had more damaging effect to the central nervous system at 40°C than at 20°C¹²⁻¹³).

In the present experiment, male Donryu rats showed less susceptibility to benzene at 10°C than at 21°C and 32°C, that is, rats given benzene in a dose of 1.0 g/kg daily showed a remarkable decrease of leucocytes at 21°C and 32°C, while no change at 10°C. This phenomenon is probably caused by the difference in phenol formation from benzene in the liver²³), and the rats exposed to a cold temperature would be minimally intoxicated by benzene.

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From another viewpoint higher temperature might cause the good circulation in experimental animals and most of the administered benzene might easily reach to the liver to be oxidized.

The mechanism of easy susceptibility to benzene in higher temperatures must be discussed in further studies.

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