DIFFERENCE IN SUSCEPTIBILITY TO BENZENE AMONG RABBIT, HAMSTER, RAT AND MOUSE

BY

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ABSTRACT

In experiments on benzene poisoning various species of animals are used, and the susceptibility to benzene differs from species to species. The special qualities of experimental animals must be well characterized in order to compare experimental results and to plan future experiments.

Four species of animals (rabbits, hamsters, rats, and mice) were used in the present experiment.

Benzene in an olive oil mixture was injected subcutaneously into the backs of the animals for five successive days. The animals were sacrified on the sixth day of the experiment. By measuring body weight, hemoglobin content, erythrocyte count, leucocyte count, spleen weight, and thymus weight of all experimental animals, the difference in susceptibility to benzene was shown obviously.

1) In mice, a marked drop in weight of spleen and thymus, and a considerable decrease in hemoglobin content were observed. In rats, body weight, weight of spleen and thymus, and leucocyte count decreased markedly, and thrombocytosis was observed. Male hamsters usually showed decrease in thymus and body weight and thrombocytosis. Rabbits showed a great decrease in weight of spleen and thymus, and a marked leucopenia and drop in body weight.

2) Rats were the most susceptible to benzene; hamsters, the least.

3) Difference in susceptibility to benzene was observed between male and female animals. Male hamsters and male mice were more susceptible than females, but on the other hand, female rats and female rabbits were more susceptible than males.

INTRODUCTION

Benzene is an excellent organic solvent which dissolves fats and oils and evaporates easily at room temperature. Furthermore, as benzene is neither acid nor alkaline and is not irritative, it is used widely in industry, for example, in binding, painting, paper smoothing and shining, in printing polyethylene and polyvinyl compounds and in other chemical processes as a synthesizing and extracting agent.

Workers suffering from poisoning in these factories using benzene,

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mainly have a chronic disturbance in the hemopoietic functions of the bone marrow, but in some special cases workers suffer from aplastic anemia.

According to the official investigation of the Ministry of Labour in March, 1959, there are 6,700 factories dealing with benzene and 44,000 labourers exposed to benzene vapour[1]. The results of the Special Health Examination given by the Ministry indicate that one fourth of these 44,000 have some pathological findings.

At present, according to Ministry of Labour Standards the benzene content in organic solvents in general is not to exceed 5%, and the working conditions have been improved[2,3]. Special Health Examinations are enforced, so that the death-rate and incidence of serious cases of benzene poisoning have decreased rapidly. However, the consumption of benzene continues to increase, so that not a few workers do suffer benzene poisoning —in some cases, aplastic anemia—particularly in small factories where supervision is not strict[4].

There are considerable individual difference in susceptibility to benzene among human beings—in one case, symptoms of benzene poisoning may appear after a few months exposure to benzene vapour, while in another case in the same environment, benzene poisoning is not found. Though the same phenomenon may also be observed in workshops using other chemical substances[5], this susceptibility difference is characteristic of benzene. Studies on the factors influencing individual differences in susceptibility to benzene have started just recently[6], so there are many unknowns in this field. For example, in studies of benzene poisoning, various kind of animals are used for experiments, we cannot, however, compare the experimental data from the various experiments as the experimental procedures are different. Further, the poisoning symptoms and the susceptibility to benzene are quite different in the various species of experimental animals. Finally, the degree of poisoning may appear different even when the same animals are used and the same dose of benzene administered, if the food given or the environmental temperature or other exprimental conditions are different[7,8].

In order to acquire the fundamental data necessary for comparison of the experimental results obtained up to now, to determine the kinds of experimental animals suitable for benzene administration and to clarify what measurements are best for determining the degree of benzene poisoning, the author carried out a series of experiments on benzene poisoning using various kinds of animals under the same conditions. He has tried to establish criteria for animal experiments on benzene poisoning in the future. The following results were obtained.

Materials and Methods

1) Materials: Four species of animals (rabbit: *Oryctolagus cuniculus*), (rat: *Rattus norvegicus*), (hamster: *Mesocricetus auratus*), (mouse: *Mus musculus*) were used as experimental animals. They were obtained from the Japanese Central Laboratory for Experimental Animals (CLEA). Since one of our previous reports[9] showed that susceptibility to benzene differs according to age, young adults were used. Rabbits of the native Japanese species (2.5–3.0 kg of body weight), rats of the Donryu strain (7 weeks of age), mice of ddN strain (6 weeks of age), and golden hamsters (9 weeks of age), 9 males and 9 females of each species, a total of 72 animals, were used for the present experiment. To make the environmental conditions as similar as possible, all animals were fed with commercial standard pelleted food. Water was given *ad libitum* in cages maintained in an air-conditioned room (temperature: 21±1°C, relative humidity: 55±5%).

2) Experimental design *in vivo*: The experimental animals of both sexes were divided into 3 groups. Each group consisted of three males and three females. A mixture of benzene and olive oil or olive oil alone was injected subcutaneously into the back of each animal of each group at the same time every morning for 5 successive days. Animals of Group A were given 0.5 ml/kg benzene, those of Group B, 1.0 ml/kg, while those of Group C received olive oil alone as a control. On the sixth day the effects of benzene on each animal were determined.

3) Items of the examination: All animals were weighed at the same time every morning[10] to evaluate the degree of benzene poisoning and to obtain data for every day management.

Blood was obtained from the animals' tails on the sixth day of the experiment, and hemoglobin content was measured by cyan-methemoglobin method[11], erythrocyte and leucocyte count by the tube method[12] and thrombocyte count by the modified Björkman method[13]. After rapid killing of the animals by blood-letting, the thymus and the spleen were weighed.

The values obtained by measurement of the noted item for each animal species were analyzed by means of a one way layout with 3 levels of benzene dose administered and 3 animals for each level. The measured values showing a significant difference are indicated in the Table 1. The susceptibility of an animal species was determined by the dose necessary to cause some change and by the number of changed items.

In the present experiment animals which showed change in many items: blood, body weight, thymus weight and spleen weight, were indeed more susceptible to benzene[15].

Table 1. Susceptibility Difference to Benzene Poisoning

	Rabbit		Hamster		Rat		Mouse	
	♂	♀	♂	♀	♂	♀	♂	♀
Body Weight						↓ 1.0		
Hemoglobin		↓ 0.5		↓ 1.0				
Erythrocyte								↓ 1.0*
Leucocyte	↓ 1.0	↓ 0.5 ↓ 1.0*			↓ 0.5 ↓ 1.0	↓ 0.5* ↓ 1.0*	↓ 0.5* ↓ 1.0*	
Thrombocyte			↑ 0.5* ↑ 1.0*					
Thymus	↓ 0.5* ↓ 1.0*	↓ 0.5 ↓ 1.0	↓ 1.0*	↓ 1.0	↓ 1.0*	↓ 0.5 ↓ 1.0*	↓ 1.0	↓ 0.5* ↓ 1.0
Spleen		↓ 0.5 ↓ 1.0*			↓ 1.0	↓ 0.5 ↓ 1.0*	↓ 0.5 ↓ 1.0*	↓ 1.0

Changes are significant at the 5% level (* 1% level), when benzene was given in a dose of figure (cc/kg/day). Dashes indicate no remarkable changes in these fields.
Upward arrows show the increase, and downward arrows the decrease, respectively.

Results

1) Body weight: After the administration of benzene, a decrease in body weight was observed in the various species of animals. An especially marked drop was seen in female rats. The greater the dose of benzene administered, the greater the decrease in body weight in general. (Fig. 1)

2) Hemoglobin content: Hemoglobin content varied according to the animal sex, and the dose of benzene, but female rabbits and male hamsters showed the greatest decrease in hemoglobin content after the administration of benzene. (Fig. 2)

3) Erythrocyte count: Generally, the greater the dose of benzene, the greater the change observed in erythrocyte count. Erythropenia was observed in all female animals. In particular, administration of 1.0 ml/kg of benzene caused a marked decrease in erythrocyte count at the 1% level of significance in female mice. (Fig. 3)

4) Leucocyte count: Marked leucopenia was observed in all animals. Even with the administration of 0.5 ml/kg of benzene there was an appreciable decrease in leucocyte count, especially in rabbits, rats, and male mice. The degree of leucopenia was different between male and female animals,

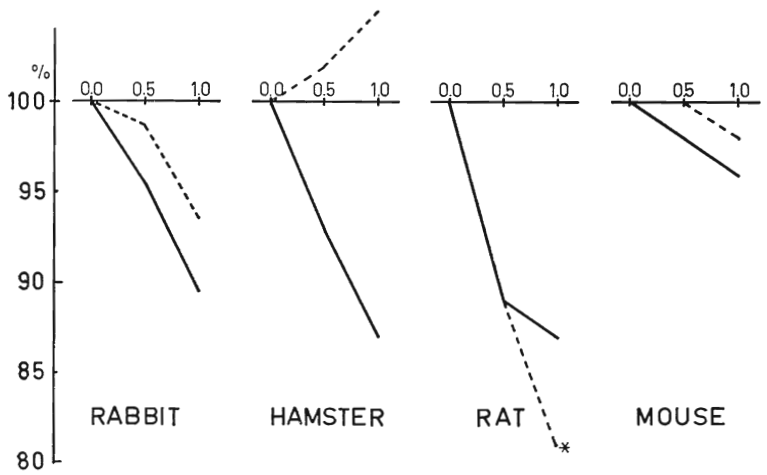

Fig. 1. Body weight
Changes are shown compared with the control in a dose of figure (cc/kg/day), and the level of significance are indicated as follows: * 5%, ** 1%. Solid lines indicate male, and divided lines female.

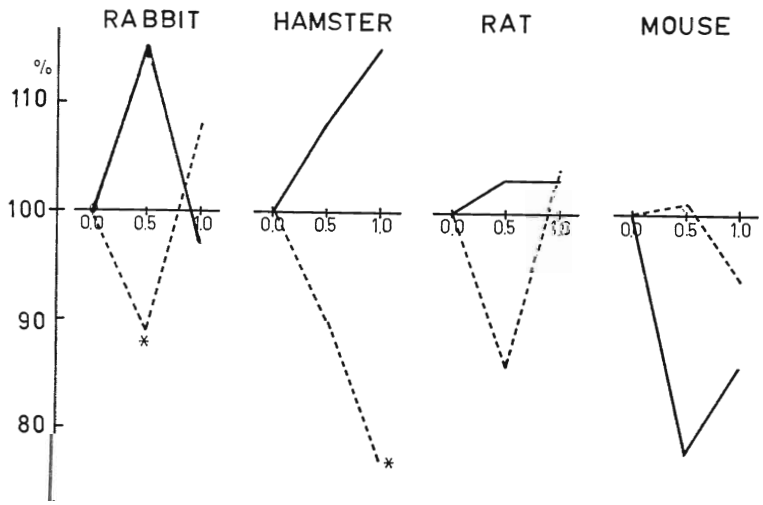

Fig. 2. Hemoglobin content

especially female rabbits and rats showed respectively more marked leucopenia than male ones. On the other hand, male mice showed a much more marked leucopenia than female mice. Thus, although females are usually more susceptible to benzene, this susceptibility may be reversed as seen in this case. Hamsters showed a smaller change in leucocyte count than other

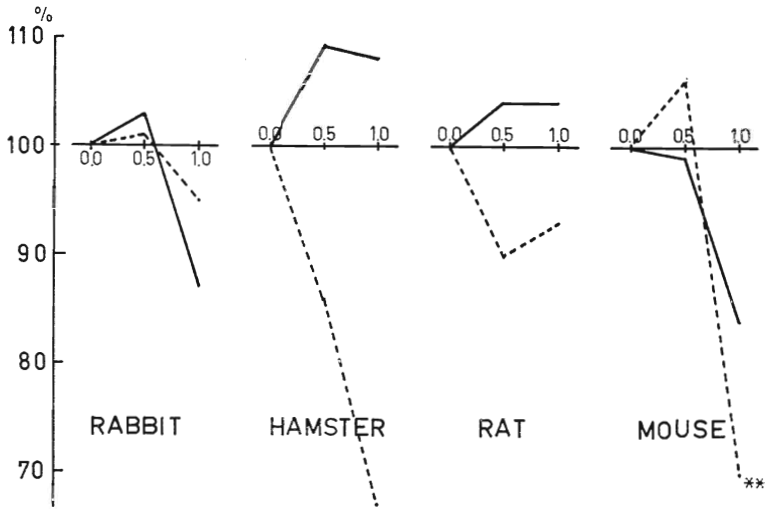

Fig. 3. Erythrocyte

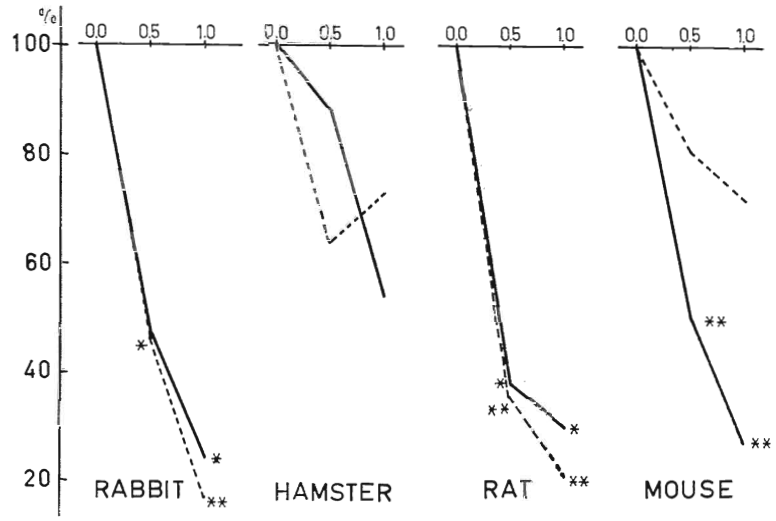

Fig. 4. Leucocyte

animals. The greater the dose of benzene administered to animals, the greater was the decrease in leucocyte count in general. (Fig. 4)

5) Thrombocyte count: Benzene brought about no appreciable alterations in thrombocyte count in general, but in male hamsters at a 0.5 ml/kg dose of benzene, the thrombocyte count increased two times, and at 1.0 ml/kg

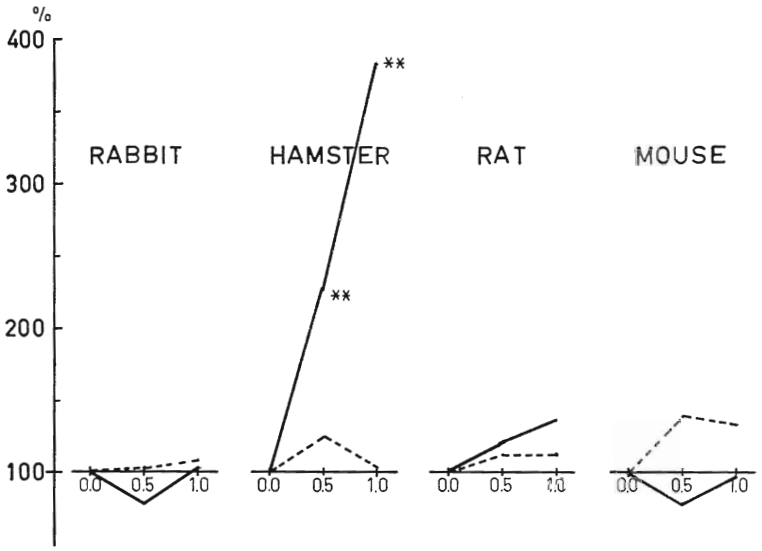

Fig. 5. Thrombocyte

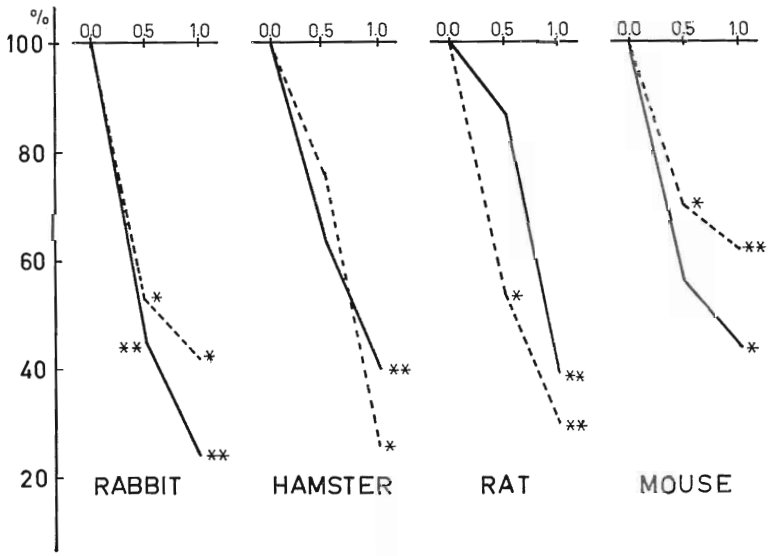

Fig. 6. Thymus weight

was four times greater than normal. (Fig. 5)

6) Weight of the thymus: There was a great loss in thymus weight in all animals. With 0.5 ml/kg a marked decrease in thymus weight was ob-

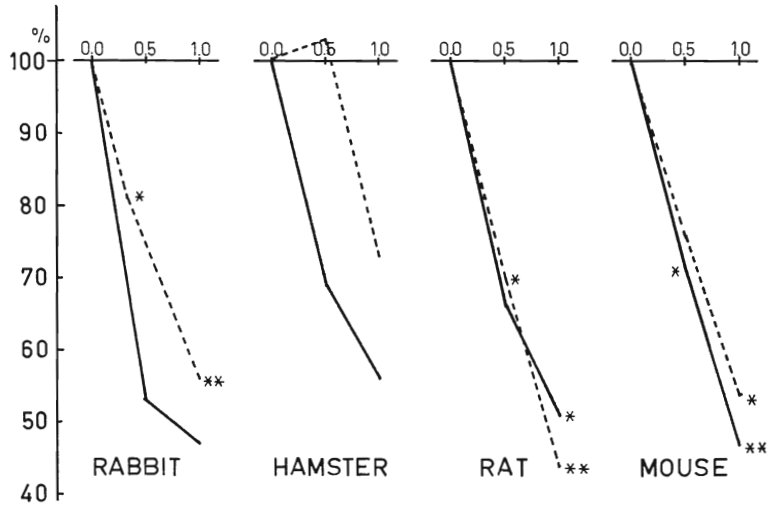

Fig. 7. Spleen weight

Table 2. Coefficient of Variation in Various Animals

	Rabbit		Hamster		Rat		Mouse	
	♂	♀	♂	♀	♂	♀	♂	♀
Body Weight	5.7% 8.6	4.7% 5.9	11.9% 13.7	9.9% 13.5	8.4% 12.1	5.5% 9.2	3.8% 5.3	4.7% 5.5
Hemoglobin	8.0 15.4	13.4 7.5	12.2	8.9	12.3 7.3	9.1 9.0	14.9 12.2	6.3 8.9
Erythrocyte	10.0 12.5	11.5 11.0	15.5	26.8	16.0 7.6	13.4 14.4	13.7 9.7	10.4 12.5
Leucocyte	30.4 47.6	29.6 43.4	31.6	61.1	19.6 52.7	43.6 18.3	24.5 25.5	25.9 67.1
Thrombocyte	24.0 19.6	21.1 14.8	12.8	17.9	14.6 16.7	9.8 26.3	9.2 15.5	19.6 20.9
Thymus	21.4	30.1	27.8	34.3	9.9	29.1	37.0	13.1
Spleen	54.7	10.9	33.6	22.2	25.2	18.0	16.6	21.9

Upper column of each field shows a coefficient of variation before the treatment, and lower column shows a coefficient after the treatment.
Vacant fields indicate no data obtained.

served especially in rabbits, mice and female rats. With a 1.0 ml/kg dose all animals showed marked decrease in thymus weight. (Fig. 6)

7) Weight of the spleen: Administration of benzene caused a drop in spleen weight in all animals of about the same degree as the drop in thymus weight. In particular the decrease in spleen weight in female rabbits, female rats, and male mice was found to be significant at the 5% level with 0.5 ml/kg doses of benzene, and a more marked loss in spleen weight significant at the 1% level was found with 1.0 ml/kg doses of benzene. (Fig. 7)

8) Coefficients of variation: In all experimental animals, the values for the coefficients of variation[16] after administration of benzene were compared with those before the experiment. Although large coefficients of variation were observed in leucocyte response in general, no significant difference in the coefficients of variation by sex or by species of animals was found. (Table 2)

Discussion

All experimental animals which were injected with benzene subcutaneously into the back for five successive days showed a decrease in body weight and in weight of the thymus and the spleen as well as leucopenia. The greater the dose of benzene administered to the animals, the greater the change observed. The degree of change varied according to the species of animal. Male hamsters showed minimal changes with 1.0 ml/kg of doses of benzene, while on the other hand, female rats showed a marked change in leucocyte count with a dose of only 0.5 ml/kg of benzene.

From the results given above, it was found that rats are the most susceptible to benzene while hamsters are least susceptible of four kinds of experimental animals. The various effects of benzene also differed by species. Rabbits showed a marked decrease in leucocyte count and in weight of the thymus and the spleen with a considerable drop in body weight. Male hamsters showed a marked decrease in body weight and in weight of the thymus and the spleen and an increase in thrombocyte count. Rats showed a marked decrease in body weight and in leucocyte counts, and in weight of the thymus and the spleen, but an increase in thrombocyte count. Mice showed a marked decrease in leucocyte count and in weight of the thymus and the spleen.

Previous experiments have also shown a marked difference among various species and even within a species. For example, ddN mice showed a marked decrease in hemoglobin content while under the same conditions CFW mice showed a marked leucopenia[17].

In this experiment rats had the most striking loss of body weight and

showed, furthermore, a marked decrease in spleen weight while, on the other hand, the most striking finding in hamsters was thrombocytosis and in rabbits leucopenia. Thus for any given experiment the criteria used for judging benzene poisoning must be chosen specially for a given species.

Females have been reported to be more susceptible to benzene poisoning than males by many investigators[18,19,20]. This difference in susceptibility to benzene poisoning according to sex occures in animals as well as in human beings. For example, female rabbits[21] and female rats[22,23] have been reported to be more susceptible than males. On the other hand, some investigators have found no difference in males and females or, as for example with mice, have found males more susceptible[9,24].

The present experiment tended to demonstrate that female rabbits, female rats, male mice, and male hamsters are more susceptible to benzene.

The above results indicate that simple comparison of experimental results of benzene poisoning is dangerous. Consideration of the data must take into account the administered dose of benzene and the species, sex and age of the experimental animals.

The reasons for the difference in susceptibility to benzene are not clear, but differences in the pathways of benzene metabolism among the various species of experimental animals may be one of the causes; injected benzene is oxidized into phenols. This oxidation process of benzene or oxides derived from benzene may bring about hemopoietic disturbances. Therefore animals with a higher oxidation rate may be expected to be more susceptible to benzene poinsoning[25,26].

It has been demonstrated in another paper[27] that even if phenols are produced in the body, animals with rapid sulphate conjugation of phenols are largely protected from benzene poisoning, so animals with a faster sulphate conjugation rate for phenols are minimally intoxicated by benzene.

With further studies of the factors leading to destruction of blood components and invasion of hemopoietic tissues by benzene and its metabolites, the reasons for the difference in susceptibility to benzene poisoning should become much clearer.

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