**STUDIES ON THE POISONING BY BENZOL AND
ITS HOMOLOGUES IV.
EXPERIMENTAL STUDIES ON THE SEXUAL DIFFERENCES
OF BLOOD PICTURE**

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It has been generally believed that women are specially liable to chronic benzol poisoning, but whether women are actually more susceptible than men appears to be uncertain, since the both sexes working under exactly similar conditions do not seem to have been investigated⁽¹⁾. In the previous report⁽²⁾, the author reported the chronic xylol poisoning of painting workers exposed to enamel thinner consisting of 40 per cent of xylol and 60 per cent of gasoline (octane, nonane, etc.). The workers of both sexes were exposed in the same conditions to xylol vapor ranging from one hundred to several hundreds p.p.m.. They were revealed to show an anemia of macrocytic type and leucopenia. The incidence of leucopenia was higher in women than in men. Since chronic xylol poisoning is generally considered to be essentially similar to chronic benzol poisoning, it may be stated that there are sexual differences of blood picture in chronic poisoning by benzol and its homologues, but the causative factors influencing the sexual differences have not been investigated.

In this report, the experiments were carried out to certify sexual differences in experimental chronic benzol poisoning of animals, and to investigate the role of the follicular hormone in the manifestations of sexual differences.

EXPERIMENTAL

Sixteen rabbits, weighing 1.8–2.5 kg, were employed, and divided into four groups (A, B, C and D) as indicated below: Group A, four males; Group B, four orchidectomized males receiving estradiol propionate; Group C, served as control; Group D, four females. The animals belonging to group B were orchidectomized under the ether anesthesia one month before the initiation of administration of benzol, and estradiol propionate was administered in 30 γ intramuscular doses per kg of body weight daily from fifteen days before the initiation of administration of benzol.

In the experimental group, i.e. group A, B and D, benzol was given by intramuscular injection of purified peanut oil containing 40 per cent of benzol in the dose of 0.5 ml per kg of body weight every two days. Therefore, these rabbits received 0.2 ml of benzol per kg of body weight every two days. In the control group, the same amount of purified peanut oil containing no benzol was given intramuscularly every two days.

Blood examinations were carried out four times during 45 days before the administration of benzol, and five times every twelve days from the 5th day to the 42nd day. Blood samples were obtained by heart puncture and examined on red and white blood cell count by Koizumi's method⁽³⁾, on hemoglobin content by copper sulfate method⁽⁴⁾, and on hematocrit by Wintrobe's method⁽⁵⁾. From these values mean corpuscular volume (M.C.V.), mean corpuscular hemoglobin (M.C.H.), and mean corpuscular hemoglobin concentration (M.C.H.C.) were calculated.

RESULTS

The body weights of experimental animals increased at almost equal rate, and there was not seen any difference among the four groups.

In group B, the effects of orchidectomy and of administration of estradiol were not observed before the administration of benzol. Before the administration of benzol, any significant findings of blood were not observed in the four groups.

To test the data statistically, the differences in square roots of any values between before and after the administration of benzol to each rabbit were analyzed by analysis of variance method.

The figures in the Tables 1-4 are the mean of the values obtained from rabbits of each group.

In the Fig. 1-6, the mean values of percentages of measurements obtained after the administration of benzol to the measurements before experiment for each group are plotted.

Red Blood Cells (Table 1 and Fig. 1): It was found that in group B and D the red cell count decreased ($p < 0.01$), and that in group D the red cell count decreased more prominently than in group A and B ($p < 0.05$) with benzol treatment. Thus the red cell count decreased by the administration of benzol and this decrease was more remarkable in the female group than in the male group and in the castrated male group receiving estradiol. It was clearly shown that there were the definite sexual differences in the decrease of red cell count of animals treated with benzol.

Table 1. Red blood cell count ($\times 10^4$)

group	before experiment	5th day	15th day	27th day	42nd day
A	578	519	525	494	519
B	545	511	471	464	500
C	633	595	594	613	640
D	564	465	468	437	473

The figures are the mean values of red blood cell counts of four rabbits.

Fig. 1. Red cell counts of four groups of rabbits. Scales are taken for the values before benzol administration as 100 in each group.

- A. males, treated with benzol
- B. orchidectomized males receiving estradiol, treated with benzol
- C. males, served as control
- D. females, treated with benzol

The following figures are illustrated similarly as in Fig. 1.

Hemoglobin Content (Table 2 and Fig. 2): In group D the hemoglobin content decreased than in group A and C ($p < 0.05$), but there was no significant difference between group B and D. Thus the hemoglobin content of the female group more decreased than that of the male group, showing the sexual difference. However, the significant difference between the female group and the castrated male group receiving estradiol was not seen.

Table 2. Hemoglobin content (g/dl)

group	before experiment	5th day	15th day	27th day	42nd day
A	14.0	12.7	12.7	12.2	13.3
B	13.7	12.6	11.3	11.6	12.1
C	15.1	14.0	13.8	14.6	14.8
D	14.2	12.2	11.5	11.1	12.1

The figures are the mean values of hemoglobin contents of four rabbits.

White Blood Cells (Table 3 and Fig. 3): It was found that there was the definite difference between group A and C, and group B and D ($p < 0.01$), and that there were not the significant differences between group A and C, and between group B and D. Thus in the female group and the castrated male group receiving estradiol, the white cell count decreased in the same manner, whereas in the male group the count did not decrease with this dose of benzol. In the white cell count, the sexual difference was clearly demonstrated.

Fig. 2. Hemoglobin contents of four groups of rabbits

Table 3. White blood cell count

group	before experiment	5th day	15th day	27th day	42nd day
A	3850	4130	3360	3540	3480
B	3600	2750	2560	2320	2480
C	5990	5890	6930	5900	6550
D	5850	5040	4440	3195	3115

The figures are the mean values of white blood cell counts of four rabbits.

Fig. 3. White cell counts of four groups of rabbits

Mean Corpuscular Volume (Table 4 and Fig. 4): M.C.V. increased in group A, B, and D ($p < 0.05$), and the increase of M.C.V. in group B and D was more prominent than in group A ($p < 0.01$). The sexual difference was also noted in the increase of M.C.V..

Table 4. Mean corpuscular volume(μ^3)

group	before experiment	5th day	15th day	27th day	42nd day
A	72.3	71.9	73.3	78.8	83.3
B	66.8	78.7	72.3	83.7	82.8
C	72.5	70.3	68.7	71.9	71.5
D	69.7	75.8	75.9	79.3	81.4

The figures are the mean values of mean corpuscular volumes of four rabbits.

Fig. 4. Mean corpuscular volumes of four groups of rabbits

Fig. 5. Mean corpuscular hemoglobin of four groups of rabbits

Fig. 6. Mean corpuscular hemoglobin concentrations of four groups of rabbits

Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration (Fig. 5 and 6): In group B and D, M.C.H. increased ($p < 0.05$), and in group B and D, M.C.H.C. decreased than in group A and C ($p < 0.05$). The effects of benzol on the M.C.H. and M.C.H.C. were found only in the female group and the castrated male group receiving estradiol.

DISCUSSION

In chronic benzol poisoning, decrease of red cell count, decrease of hemoglobin content, decrease of white cell count, increase of mean corpuscular volume, increase of mean corpuscular hemoglobin and increase of mean corpuscular hemoglobin concentration were noted in the literature⁽⁶⁾. These signs were also found in the female group of this experiment, except mean corpuscular hemoglobin concentration which showed the adverse result, i.e. the decrease.

The data obtained in this experiment demonstrate that the female was more susceptible than the male to chronic benzol poisoning, and hemoglobin content and white cell count of the male were not affected by the dosage of benzol used here. By the daily dosages of 0.2 or 0.4 ml of benzol per kg of body weight which corresponds to two- or four-folds of the dosage used in this experiment, red and white cell count and hemoglobin content decreased seriously in the male rabbits⁽⁷⁾. It seems necessary to use the adequate dosage of benzol for the observation of the sexual difference. The sexual differences of blood picture which have been noted in chronic poisoning by benzol and its homologues in industrial workers were also clearly shown in these experimental animals.

It must be mentioned that in the castrated males receiving estradiol the white cell count and M.C.H.C. decreased and M.C.V. and M.C.H. increased similarly as in the females, and that the red cell count and hemoglobin content of the former seemed to be lower than in the males, although not statistically significant. Thus, it is suggested that the follicular hormone play a important role in the chronic benzol poisoning. The high susceptibility of women to chronic poisoning by benzol and its homologues may be attributed to the effect of the follicular hormone. Accordingly, it is supposed reasonable that women are attacked severe by exposure to benzol and its homologues in their younger ages.

However, the mechanism of action of the follicular hormone on chronic benzol poisoning remained obscure.

SUMMARY

An experimental study on chronic benzol poisoning was carried out. Benzol was administered in the dose of 0.2 ml per kg of body weight every two days. Four males, four orchidectomized males receiving estradiol, and four females were treated with benzol, and four males were served as control.

Females were more liable to chronic benzol poisoning than male. Concerning blood findings, the castrated males receiving estradiol responded in the similar

way as in the females to benzol treatment. The high susceptibility of women to chronic poisoning by benzol and its homologues may be explained by the effect of the follicular hormone.

REFERENCES

- 1) Browning, E.: Toxicity of industrial organic solvents, Revised Ed. p. 32, Her Majesty's Stationary Office, London, 1953.
- 2) Hirokawa, T.: Some findings on the chronic xylol poisoning. *J. of Science of Labour*, 30, 381-389, 1954.
- 3) Koizumi, A.: A new method of blood count—Studies on the application of clinical tests to the groupexamination. (1). *Nisshin Igaku (Jap. J. Medical Progress)*, 38, 252-254, 1951.
- 4) Yoshikawa, H.: Ryusando-ho (Copper sulfate method). p. 3-65, Tokyo Shuppan & Co., Tokyo, 1948.
- 5) Wintrobe, M. M.: Clinical hematology, p. 242-244, Lea & Febriger, Philadelphia, 1949.
- 6) Greenburg, L., Mayers, M. R., Goldwater, L., and Smith, A. R.: Benzene (benzol) poisoning in the rotogravure printing industry, in New York City. *J. Indust. Hyg. & Toxicol.*, 21, 395-420, 1939.
- 7) Ueoka, D.: Influence of the nutrients on the blood components of the benzol administered rabbits. *Jap. J. Nation's Health*, 22, 24-40, 1953.