# THE ACTION OF BENZOL*

## VI. BENZOL VAPOR LEUCOPENIA (RABBIT).

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Experimental work on the action of benzol was the outcome of the occurrence of cases of poisoning in various industrial establishments where benzol was used.

In 1910 Selling[1] reported a series of cases of benzol poisoning occurring in girls employed in one room of a factory for the manufacture of tin cans. In this room rubber dissolved in benzol was used as a substitute for solder. The atmosphere of the room had a distinct odor of benzol, and it was believed that the chief absorption of the benzol was through the lungs. The chief clinical features of these cases were purpura hemorrhagica, leucopenia and anemia.

Selling[2] later, working on rabbits, reports that "attempts were first made to produce chronic poisoning by inhalation, but as these resulted unsuccessfully, recourse was had to subcutaneous injections." No mention of the technic used in the inhalation experiments is made.

White and Gammon[3] worked with inhalations of benzo in rabbits. They say, "Inhalations were given from a wide-mouthed glass staining bottle with absorbent cotton in the bottom of the bottle. The mouth of the bottle was held lightly over the mouth and nose of the rabbit so that very little benzol gas was wasted." As a result of this work and his later work, White says:[4] "We are now in a position to say, however, that we have not been able to produce in well rabbits any anemia or leucopenia by inhalations of benzol alone."

In the technic used by us, the rabbits were kept in an atmosphere containing vaporized benzol, and no attempt was

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made to distinguish between inhalation and other possible methods of absorption.

The action of benzol is such as to give it great importance in certain anatomical and physiological studies. For this reason, as well as on account of the industrial importance of benzol poisoning, the reported unsuccessful attempts to produce similar poisoning in rabbits by inhalation, and for the sake of comparison with the results of other methods of administration, rabbits were exposed to benzol vapor under conditions somewhat comparable to those existing in industrial establishments, and the results were studied. Special attention was given to the blood cell counts.

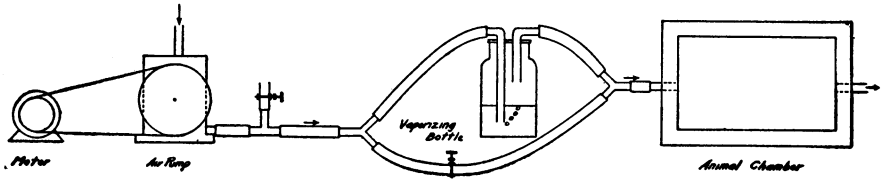


FIG. 1.—Apparatus for the exposure of rabbits to benzol vapor.

The apparatus used as illustrated in Fig. 1. The animal chamber is 50 cm. long, 23.5 cm. wide and 26 cm. high. It has glass sides and top, so that the condition of a rabbit in the interior could be readily observed at any time. The air pump was driven by a 1-12 h.p. electric motor. By tightening or loosening the clamp on one arm of the Y located at the end of the tube leading from the pump, the amount of the air which was driven through the benzol in the vaporizing bottle could be increased or decreased. The Y inserted beyond the vaporizing bottle served to mix the air which had passed through the benzol with that coming directly from the pump. The clamp on the T located between the pump and the first Y was always kept completely closed, and consequently was of no effect.

Maximal sublethal dosage was desired, and consequently no attempt was made to make accurate determinations of the degree of concentration of the benzol in the chamber. However, the average amount of benzol vaporized per hour was 16.6 cubic centimeters, — 17.4 cubic centimeters for the ten-hour rabbits and 15.9 cubic centimeters for the twenty-four hour rabbits mentioned below. A gas meter was temporarily incorporated in the apparatus between the second

Y and the animal chamber, and it was found that 71.4 liters of air and vaporized benzol per hour was being pumped into the chamber.

Eleven rabbits were used. Table VII. contains data in regard to them. They were kept in individual cages under constant conditions for a time before being placed in the chamber. In the table, under the heading "Months in

TABLE VII.

*Data of rabbits (Belgian hares) used.*

| Animal No. Experiment. | Pedigree. | Born. | Age, Months. | Sex. | Weight in Grams before Exposure. | Months in Individual Cage. | Exposure Begun. |
|---|---|---|---|---|---|---|---|
| 17.6 | 1 x 3 | 1- 5-'17 | 10 | Male. | 3,330 | 6 | 11-19-'17 |
| 17.7 | 1 x 4 | 2-26-'17 | 9 | Male. | 2,540 | 2 | 12- 3-'17 |
| 17.8 | 1 x 3 | 1- 5-'17 | 11 | Female. | 3,490 | 11 days. | 12-10-'17 |
| 17.9 | 1 x 4 | 2-26-'17 | 9 | Male. | 2,560 | 3 | 1-14-'18 |
| 18.1 | 1 x 3 | 4-29-'17 | 11 | Male. | 3,060 | 6 | 4- 8-'18 |
| 18.2 | 1 x 3 | 4-29-'17 | 11 | Male. | 3,190 | 6 | 4-18-'18 |
| 18.3 | | 5- -'16 | 25 | Male. | 3,630 | 17 | 6-10-'18 |
| 18.4 | | 5- -'16 | 25 | Female. | 2,900 | 18 | 7- 1-'18 |
| 18.5 | 1 x 3 | 4-29-'17 | 14 | Male. | 2,930 | 9 | 7- 5-'18 |
| 18.6 | 1 x 4 | 2-26-'17 | 17 | Female. | 2,700 | 8 | 7-22-'18 |
| 18.7 | 1 x 3 | 4-29-'17 | 15 | Female. | 3,005 | 8 | 8- 2-'18 |

Individual Cage," is given the length of this time for each rabbit. During the whole course of the experiments they were kept in these same cages when not in the chamber.

During the summer the rabbits were fed exclusively on grass, oats and water; during the winter, exclusively on carrots, oats and water. The changes in diet were made on about the first of June and the first of October. No changes

in the results of blood counts or of any of the other forms of observation referable to changes in diet were observed.

Six of the rabbits were kept in the chamber, with the apparatus running, for ten successive hours daily. Five were exposed continuously, being kept in the chamber continuously, except for a few minutes each morning, when they were removed for the purpose of weighing and obtaining specimens of blood for counting. Such continuous exposure was used in an attempt to produce a more rapid and marked leucopenia than that produced by the discontinuous ten-hour exposures. However, these rabbits showed changes which differed in no essential from those occurring in the rabbits exposed for only ten hours daily, and will therefore not be considered separately.

Leucocyte counts were made daily in the forenoon. Blood smears were made, and the rabbits were weighed each time the leucocytes were counted.

Leucocyte counts were made on at least three successive days (average five), immediately before exposure was begun. Immediately after these counts, as a control, seven of the rabbits were each kept in the chamber with apparatus running for a time with no benzol in the vaporizing bottle, and consequently with no benzol vapor present in the air entering the chamber; two for four days, three for three days, and two for two days. The observations during these periods showed no changes that could be attributed to their presence in the chamber. As pointed out below, changes due to the benzol vapor began within two days. Therefore the changes described below were due to benzol vapor.

Some of the rabbits were allowed to survive for a considerable time after exposure had been discontinued. The daily forenoon leucocyte counts on these were continued until the leucocyte curve had apparently assumed a permanent level after the discontinuance of exposure; then, for a time, they were made twice a week. If there was still no apparent change in general level, they were then made at weekly intervals.

Erythrocyte counts were made once in two days. After exposure had been discontinued for some time, and the erythrocyte curve had apparently assumed a permanent level, they were made at weekly intervals.

Exposure was continued until the rabbit stopped eating, and showed evidences of extreme weakness. Then, in case the period of exposure was not completed, the concentration of the benzol vapor was reduced until the rabbit was out of danger. In general, maximal sublethal dosage was attempted. Two of the rabbits died on the first day, one on the second and one on the third day of exposure. Three, apparently dying, were killed; one on the fourth day, one on the sixteenth day, and one on the sixty-ninth day after exposure was begun; the two latter, as shown later in Table VIII., survived until the fifth and sixteenth days, respectively, after exposure was discontinued. Four survived, and observations on them were continued for an average of three hundred sixty-eight days after the leucocyte curve had risen to a permanent general level after exposure, — in one case four hundred fifty-eight days.

The leucocyte curve. — The general trend of the curves may be followed in Chart 13 and Table VIII. In every instance but one, the curve began to fall within two days after the beginning of exposure. In this one instance (Experiment 18.5) it showed no fall until the fifth day. In an average of six days after the beginning of exposure, the curve fell sharply to a general low level. After this, with continued exposure, there were fluctuations in the level which seem to be largely dependent upon daily variation.

In Table VIII. is given, for each rabbit, the number of days after the beginning of exposure, when the general low level was reached. This was not necessarily the day on which the " Lowest Level Reached " fell, because, during subsequent daily variation, a still lower level was in some cases reached. The average lowest level reached is 1,768 as compared with an average of 11,892 before exposure.

The fall was thus just as sharp as under the conditions of our experiments dealing with subcutaneous injections of olive oil-benzol mixture.[5, 6] However, the curve never fell as low as 1,000, although in one case exposure was continued over a period of fifty-three days. The lowest level reached in any case was 1,260. If an attempt was made to lower the curve more by increasing the concentration of the benzol

TABLE VIII.

*Data in regard to leucocyte curves of rabbits exposed to benzol vapor.*

| Rabbit No. Experiment. | Average before Exposure. | Beginning Fall in Days. | Time in Days of Arrival at General Low Level. | Lowest Level Reached. | Duration after Exposure, of General Low Level. | Time in Days, after Last Exposure, of End of Rise to Permanent Level. | Average for One Year after End of Rise to Permanent Level. | Daily Exposure in Hours. | Exposure in Days. | Dying, Killed. |
|---|---|---|---|---|---|---|---|---|---|---|
| 17.6 | 10,470 | 2 | 6 | 1,800 | 36 | 44 | 6,500 | 10 | 6 | |
| 17.9 | 13,940 | 1 | 9 | 1,380 | | | | 10 | 53 | 69th day. |
| 18.2 | 11,167 | 2 | 6 | 1,780 | 23 | 51 | 6,500 | 10 | 24 | |
| 18.5 | 11,613 | 5 | 8 | 1,260 | | | | 24 | 11 | 16th day. |
| 18.7 | 12,270 | 2 | 3 | 2,620 | 12 | 36 | 5,500 | 24 | 11 | |
| Average | 11,892 | | 6.4 | 1,768 | | | 6,166 | | | |

vapor the rabbits stopped eating, showed evidences of extreme weakness and were in apparent danger of dying; and in no case have we been able to lower the leucocyte curve to or below 1,000, as may be done by means of injections.

The fact that the rabbits exposed continuously showed no more marked changes than those occurring in the rabbits exposed for only ten hours daily apparently demonstrates that the effect produced in these experiments is the maximum effect which it is possible to obtain by this method of administration.

After exposure was discontinued and its immediate effect had ceased, the curve remained at the general low level for periods varying from twelve to thirty-six days in the individual rabbits (Table VIII.). Then it rose gradually to a permanent general level, arriving at this level at a time varying from thirty-six to fifty-one days after the discon-

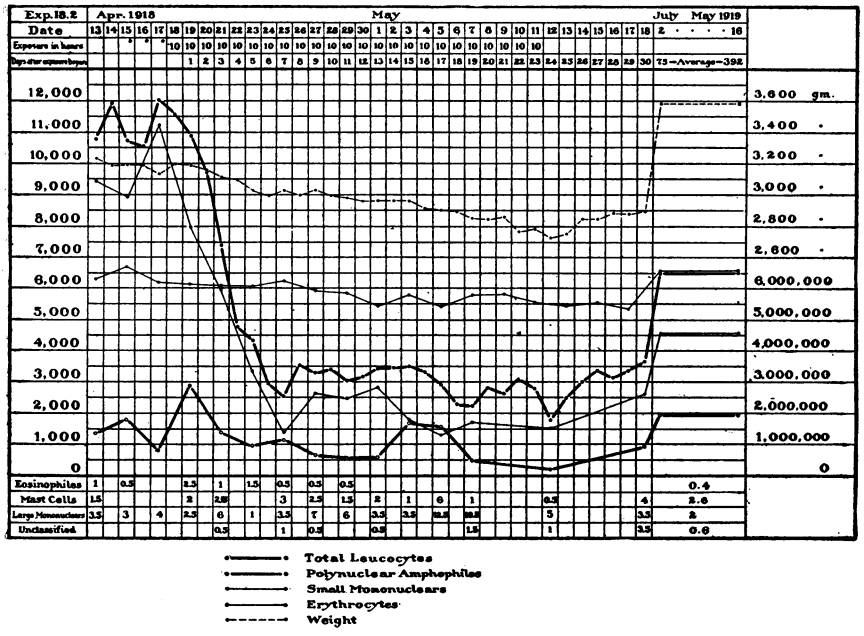


Chart 13. (Exp. 18.2). — Total leucocyte, absolute polynuclear amphophile, absolute small mononuclear, erythrocyte and weight curves.

In chamber, apparatus in operation with no benzol in vaporizing bottle.

tinuance of exposure. This permanent general level was considerably lower than that existing before exposure was begun. This lower permanent level was maintained as long as counts were continued, and in no instance did the curve return to a level as high as the lowest level observed before exposure was begun, but there occurred only irrelevant daily variations, and other irregular periodic variations to which

the total leucocyte curve of the non-exposed rabbit is probably subject. In the case of the three tabulated rabbits on which counts were continued for a period of a year after the permanent general low level was reached, the average level of the curve before exposure was 11,302, while the average lower permanent general level was 6,166. One additional rabbit (Experiment 18.3) showed this same difference, but is not included in Table VIII. on account of being atypical in other parts of the curve. In the four possible instances the difference was thus shown without exception. It is therefore apparent that the rabbits were in some way permanently affected by exposure, with the result that the leucocyte count was markedly and permanently lowered. As pointed out above, they were kept under constant conditions from a time considerably before exposure up to the time of autopsy, and appeared perfectly well after recovery from the immediate effects of exposure. The results of these observations would be more convincing if the leucocyte counts before exposure also covered a period of one year instead of only a few successive days. It is probable that the level of the leucocyte curve of non-exposed rabbits is subject to considerable irregular periodic variation.

Following the rise in the leucocyte curve after exposure was discontinued, there occurred in no case any evidence of a secondary fall (deuterophase) such as occurred after injections. However, it should be pointed out that in only one case (Exp. 17.6) was exposure discontinued as soon as a low level was reached, as was done in the injection cases.

The absolute differential leucocyte curves. — The following conclusions were drawn in the fifth article of this series[7] :

"... Most of the polynuclear cells of normal rabbit's blood are amphophiles; very few of them are eosinophiles and mast cells. ... Most of the mononuclears are small mononuclears; a few of them are large mononuclears. ... The classification of the leucocytes into these two groups will give results which represent essentially the behavior of the polynuclear amphophiles and the small mononuclears, and, although the figures which we shall present include all polynuclear cells in the one case and all mononuclear cells in the other, yet we shall use the terms amphophile and small mononuclear."

The data on which these conclusions are based are given in full in the fifth article.[7] This classification will be used here. The number of unclassified cells (average in all the rabbits, 0.9 per cent) was not sufficiently large to have any important bearing upon the interpretations here given.

Absolute differential counts were made on all rabbits covering the same period as was covered by the total leucocyte counts. The intervals at which they were made may be judged from Chart 13. During the period of the permanent low general level they were made once a month, and at times more frequently. Curves based on these absolute counts were plotted. Important points in the curves are tabulated in Table IX., and the curves of one of the rabbits may be followed in Chart 13.

Before exposure, the percentage differential counts showed that the average percentage of amphophiles was 16.5, and of small mononuclears was 82.5. The average total leucocyte count was 11,892 (Table VIII.). Consequently the average absolute amphophile count was 1,959 and the average absolute small mononuclear count was 9,813 (Table IX.).

In all cases the fall in the total leucocyte curve after the beginning of exposure is due largely to a fall in the absolute small mononuclear curve, the average loss of small mononuclears being 8,714 and the average loss of amphophiles 1,298. The average percentage loss of small mononuclears was 89, that of amphophiles was 66.

In the rabbits on which relevant observations were made as described above, the permanent low general level of the total leucocyte curve resulting from exposure is due to a failure of the absolute small mononuclear curve to return to as high a level as that existing before exposure (Table IX. and Chart 13). The average absolute small mononuclear count before exposure was 9,813, as compared with an average of 4,277 for one year after the rise to a permanent level following the discontinuance of exposure. The average absolute polynuclear amphophile counts for these same periods were 1,959 and 1,883, respectively.

In the injection cases[7] (p. 493) it was also found " that the lower average level of the total leucocyte curve at the end of the protophase and at the end of the deuterophase " of the diphasic leucopenia, " as compared with that existing before injections, is dependent entirely on the lower average level of the small mononuclear curve." A reëxamination of these total leucocyte curves in the injection experiments shows that they tend to rise soon to the original level and to show no

TABLE IX.

*Absolute differential counts at various stages.*

| Animal No. Experiment. | Average before Exposure. | | Lowest Level Reached. | | Average for One Year after End of Rise to Permanent Level. | |
|---|---|---|---|---|---|---|
| | Polynuclear Amphophiles. | Small Mononuclears. | Polynuclear Amphophiles. | Small Mononuclears. | Polynuclear Amphophiles. | Small Mononuclears. |
| 17.6 | 2,144 | 8,177 | 1,476 | 306 | 1,489 | 5,011 |
| 17.9 | 1,500 | 12,136 | 400 | 980 | | |
| 18.2 | 1,316 | 9,851 | 231 | 1,549 | 1,933 | 4,547 |
| 18.5 | 1,874 | 9,665 | 724 | 510 | | |
| 18.7 | 2,961 | 9,234 | 472 | 2,148 | 2,228 | 3,272 |
| Average | 1,959 | 9,813 | 661 | 1,099 | 1,883 | 4,277 |
| Average Total Leucocytes | 11,892 | | 1,768 | | 6,166 | |

persisting effect of the injections. It appears thus that the results of the two methods of administration differ from each other in this respect.

In Chart 13 we have tabulated the percentages, as exactly as possible, of eosinophiles, mast cells and large mononuclears, on each of the days involved in the plotted curves of the absolute amphophile and small mononuclear counts. The percentages of unclassified cells are also given. Blank spaces

signify that no cells of the kind involved were found. Counting two hundred cells, the daily percentages do not change in any apparently significant way.

The erythrocyte curve. — The rabbits tabulated relative to the leucocyte curve in Tables VIII. and IX. are tabulated relative to the erythrocyte curve in Table X. After the beginning of exposure, the erythrocyte curve fell gradually to a general low level (Chart 13). This general low level was reached in from six to sixteen days. Afterward this general low level was maintained as long as exposure was continued; in one case, thirty-seven days. There was no tendency for the curve to go lower after the general low level was once reached. After the discontinuance of exposure, the curve gradually rose to the level existing before exposure, arriving at this level in from fifteen to twenty-four days.

The most marked fall was from 6,203,000 down to 4,236,000. The average fall was from 6,126,750 down to 4,947,000. The degree of fall corresponds to that observed in the injection experiments.[5] In the injection experiments, however, the conditions were such that injections were not continued after the corresponding low level was reached.

The behavior of the erythrocyte curve we regard as corresponding to its behavior in intoxications in general, and not as corresponding to the extreme grades of anemia observed in cases of industrial benzol poisoning.

The weight curve. — After the beginning of exposure the weight curve began to fall in all the rabbits tabulated in Tables VIII., IX. and X. (Chart 13). The fall continued as long as exposure was continued except in one rabbit. In this one (Exp. 17.9) it fell for eight days and then remained stationary for all except the last five of the other forty-five days of exposure. The slowest fall was shown by rabbit Experiment 18.2 (Chart 13) in which four hundred twenty-five grams were lost in the twenty-three days of exposure. The most rapid fall was shown by rabbit Experiment 18.5, in which eight hundred grams were lost in the

eleven days of exposure. In the three surviving rabbits the curve began to rise within two days after the discontinuance of exposure. This rise, which was gradual, continued for about one month, at which time the curve reached, or nearly reached, the level existing before exposure; and afterward it maintained this level or rose above it. In the two that were killed when apparently dying, the curve fell very rapidly for a number of days before death.

TABLE X.

*Data in regard to erythrocyte curves of rabbits exposed to benzol vapor.*

| Rabbit No. Experiment. | Average before Exposure. | Time in Days of Arrival at General Low Level. | Lowest Level Reached. | Duration of General Low Level to End of Exposure. | Time in Days, after Last Exposure, of End of Rise to Original Level. | Daily Exposure in Hours. | Exposure in Days. | Dying, Killed. |
|---|---|---|---|---|---|---|---|---|
| 17.6 | 6,198,000 | 8 | 5,540,000 | | 15 | 10 | 6 | |
| 17.9 | 6,203,000 | 16 | 4,236,000 | 37 | | 10 | 53 | 69th day. |
| 18.2 | 6,396,000 | 13 | 5,348,000 | 11 | 17 | 10 | 24 | |
| 18.5 | (5,418,000) | | | | | 24 | 11 | 16th day. |
| 18.7 | 5,710,000 | 6 | 4,664,000 | 5 | 24 | 24 | 11 | |
| Average | 6,126,750 | 10.7 | 4,947,000 | | | | | |

Six of the rabbits are not included in Tables VIII., IX. and X. Of these, two died within two days, three between two and four days after the beginning of exposure, and one survived. The two dying within two days showed no changes in the total leucocyte, absolute differential or erythrocyte curves which could be attributed to exposure. The three dying between two and four days all showed a fall in the leucocyte curve which in one case reached 1,800. They showed a characteristic fall of the absolute small mononuclear curve, but the amphophile curve showed a tendency toward

rather marked, irregularly alternating, daily rises and falls. However, these rabbits are not included in the tables because of the fact that death probably occurred before the maximum effect upon the leucocytes was produced. There was no fall in the erythrocyte curve. The weight curve fell very rapidly. The surviving rabbit (Exp. 18.3) showed a fall of 1,000,000 with a corresponding subsequent rise in the erythrocyte curve, but an atypical leucocyte curve during exposure, in that there occurred marked, irregularly alternating daily rises and falls, due entirely to increases and decreases in the number of amphophiles. After the discontinuance of exposure, however, the curve followed a course corresponding to that of the three tabulated surviving rabbits. Its weight curve fell and rose characteristically.

Autopsies. — Careful and thorough autopsies were made in all cases. Of the seven rabbits which died as a result of exposure — or were killed when apparently dying — two died on the first day and showed edema of the lungs. Four of the other five showed hemorrhages: three, hemorrhages into the gastro-intestinal tract, and one, hemorrhages into the lungs and a few small ulcers in the gastric mucosa. The other showed apparently a few small gastric ulcers. One showed tarry stools before death.

The results of histological examination will not be considered in this article.

These results show that in rabbits, during exposure to benzol vapor under the conditions described above, there occurs marked leucopenia. The occurrence of hemorrhages and tarry stools in rabbits dying as a result of exposure represents the occurrence of a purpuric condition. Slight anemia occurs.

Examination of the results show that they are of the same general nature as those produced by subcutaneous injections of olive oil-benzol mixture. The question of the non-occurrence of diphasic leucopenia in the benzol vapor experiments may perhaps profitably be taken up in connection with the results of histological examinations.

## CONCLUSIONS.

1. Exposure of rabbits to benzol vapor with maximal sublethal dosage causes leucopenia, hemorrhages and slight anemia.

2. The percentage and absolute decrease of small mononuclears is greater than that of polynuclear amphophiles.

3. Apparently, after discontinuance of exposure, the total leucocyte curve rises to a permanent general level, lower than that existing before exposure. This relative leucopenia is permanent. It is due to a failure of the absolute small mononuclear curve to rise to as high a level as that existing before exposure.

4. The results of exposure are of the same general nature as those produced by subcutaneous injections of olive oil-benzol mixture. Diphasic leucopenia was not observed.

[The air pump used in the experimental work was loaned by the Nutrition Laboratory of the Carnegie Institution of Washington, through the kindness of Dr. H. Monmouth Smith.]

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