# THE INACTIVATING EFFECT OF SULFAPYRIDINE ON THE LEUKOTOXIC ACTION OF BENZENE*

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PLATE 24

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In an attempt to study *in vivo* the rôle of leukocytes in the curative action of sulfonamide drugs for infections, leukopenia was produced in rabbits by the administration of benzene. During the period of intoxication the leukopenic animals were infected with pneumococci and treated with sulfapyridine. Numerous difficulties arose which rendered the experiments indeterminate, and in the course of exploring the difficulties it became apparent that sulfapyridine exerted an inhibitory effect on the toxicity of benzene for the leukopoietic system of rabbits. The data arising from an investigation of the relationship between sulfapyridine and benzene comprise the basis for the present report.

## *Methods and Materials*

The experimental procedure consisted primarily of determining by daily estimations the number of leukocytes in the circulating blood of rabbits receiving sulfapyridine simultaneously with injections of benzene, and also in other rabbits receiving benzene alone. The observations have been extended to include additional sets of animals, some of which received sulfapyridine alone, others received *p*-aminobenzoic acid together with benzene, and still others consisted of a normal untreated group.

For comparative purposes, therefore, five groups of rabbits were employed, as follows:

*Group 1.* *Twenty-six* rabbits received benzene alone. In each experiment, two or more animals of this group were included.

*Group 2.* *Twelve* rabbits received both sulfapyridine and benzene.

*Group 3.* *Seven* rabbits received sulfapyridine alone.

*Group 4.* *Six* rabbits received *p*-aminobenzoic acid together with injections of benzene.

*Group 5.* *Eleven* normal, untreated rabbits were also followed.

In addition to observations on the quantitative behavior of the leukopoietic system during the periods of the several treatments, determinations of the urinary excretion of phenols have been made in a limited number of experimental animals. It has long been known that in the animal body benzene is oxidized to form phenols (1). Kracke (2, 3) has made observations on the oxidation of benzene in rabbits, and he

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advanced the theory that one or more of the phenolic oxidation products may be responsible for the leukotoxic action rather than the unchanged benzene itself. In the preliminary studies, included in the present report, of a possible chemical explanation of the effect of sulfapyridine on the toxicity of benzene, the excretion of phenols in the urine has been used as an index of the extent of oxidation of benzene to phenolic products.

Adult rabbits weighing about 2.5 kilos were uniformly employed. Leukocyte counts were made each day at the same hour in order that the uncontrolled variables of the white blood cells in the peripheral blood would be reduced to a minimum. Differential counts of stained smears were also made in a limited number of the rabbits. The blood used in making the determinations was derived from the marginal ear veins.

*Injections of Benzene.*—The original procedure of Selling (4) for the dosage and route of injection of benzene was employed. A daily dose of 5.0 cc. of a mixture of equal parts of benzene and olive oil (approximately 1.0 cc. of benzene per kilo of body weight) was injected subcutaneously beneath the skin of the abdomen. A new site was selected for each injection in order to avoid introducing the mixture into an area of edema or inflammation resulting from a prior injection of benzene.

*Administration of Sulfapyridine, per Os.*—Sodium sulfapyridine monohydrate in 5 per cent solution was administered by stomach tube in two equal doses of 1.25 gm. at 9 a.m. and 5 p.m. each day, thus giving a total daily dose of approximately 1.0 gm. per kilo. The benzene was injected at noon, and consequently animals which received both substances were given their first dose of benzene 3 hours after the initial dose of sulfapyridine. Although at the beginning the stomach tube was inadvertently introduced into the trachea, after brief experience the procedure proved simple and the animals evidenced no immediate untoward reaction. No vomiting occurred. The sodium salt was used in order to facilitate the administration of the drug and to insure maximum adsorption.

*Administration of p-Aminobenzoic Acid, per Os.*—*p*-Aminobenzoic acid was given by stomach tube as a 5 per cent solution of the sodium salt. Two different dosages were used: 0.5 gm. twice daily and 1.0 gm. twice daily.

*Determination of Urinary Phenols.*—The determinations of urinary phenols were done by the method of Folin and Denis (5) using the modified phenol reagent of Folin and Ciocalteu (6, 7). Briefly described, the method consisted of a colorimetric comparison of a filtrate of the urine with a standard solution of phenol after each had been treated with a phosphomolybdic-phosphotungstic reagent. Interfering substances (uric acid, protein) were first removed from the urine by the precipitation with silver lactate and a colloidal iron solution, and the excess silver was precipitated with chloride. The concentration of "free" phenols was obtained from a direct determination on the filtrate, and the concentration of total phenols was found by a similar determination carried out after hydrolysis of the filtrate with hydrochloric acid. The difference between total phenols and "free" phenols represented the conjugated phenol fraction.

## EXPERIMENTAL

*Group 1. Effects of Benzene Alone.*—Selling (4) made the observation that, following the daily injection of 2.0 cc. per kilo of the benzene-olive oil mixture,

the number of circulating leukocytes was sharply reduced after a few days, and that the decrease continued progressively until, after continued injections, the animals died with an extreme degree of leukopenia. If the count reached 40 to 720 cells per c. mm., the animals usually died despite the suspension of injections of benzene. Study of the bone marrow revealed that this was the primary seat of the damage rather than the destruction of mature leukocytes in the circulating blood. There was some evidence of destruction in the marrow after 2 days and practically complete aplasia was found after about the ninth injection. The leukopenic effect resulting from intoxication by benzene has been uniformly experienced by many investigators, notably by Kline and Winternitz (8), Weiskotten and his coworkers (9), Camp and Baumgartner (10), Rich and McKee (11), and others.

In the present studies normal rabbits, receiving injections of benzene, have served as controls in each group of experimental animals. The uniformity of the leukopenic effect is attested by the results obtained with 26 rabbits. These rabbits received a daily injection of 5.0 cc. of the mixture of equal parts of benzene and olive oil, and white blood cell counts were made daily. A composite curve derived by averaging the counts obtained in all 26 rabbits is presented in Chart 1. The results indicate the rate of development and severity of the leukopenia. The curve shows an initial rise after the first injection which is in turn followed by a rapid fall during the succeeding 6 days. More specifically, the initial pretreatment average of 8900 rose to 10,200 after 1 day and then fell to 6700 after 2 days, 4700 after 3 days, 3800 after 4 days, 2100 after 5 days, 1500 after 6 days and 880 after 7 days. When the daily injections were continued, death regularly ensued in 12 days or less (average approximately 8 days) and the leukocyte count usually fell below 100.

Although there was some variation in the rapidity with which the leukopenia developed in the individual rabbits, the composite curve gives a reliable picture of the usual trend of the leukocyte count. The count reached a level of 1000 cells per c. mm. or below in from 3 to 9 days in all but one of the animals, and this single exception died on the 5th day with a count of 2200.

*Group 2. Effects of Benzene and Sulfapyridine.*—Twelve animals were injected with benzene according to the regular daily schedule and in addition received sulfapyridine, by oral administration, coincidentally with the benzene. They were maintained on this regime for periods varying from 6 to 19 days. The detailed findings of the individual animals in this group are presented in Table I; the average of results is given in Chart 1. The composite curve of the average of the leukocyte counts of the twelve rabbits (Chart 1) when contrasted with that of the rabbits who received benzene alone reveals the sparing effect of the sulfapyridine on the benzene intoxication. The curve of the group receiving the combined treatment exhibited no sustained downward trend. There were, however, daily fluctuations which were greater than those

seen in normal, untreated rabbits (compare with curve of 11 normal rabbits, Chart 1). A comparison of the initial, pretreatment count with the average counts of the final 3 days of treatment (days 9 to 11 in the chart) reveals that the difference is slight. It will be observed that there is a definite increase in the average counts on the first 3 days after the beginning of treatment, and that the lowest point on the curve, reached on the 8th day, is at 8800 which is only 1500 below the initial count.

TABLE I

*Daily Determination of the Number of Leukocytes in the Blood of Rabbits Receiving Sulfapyridine and Benzene Simultaneously*

| | Days after beginning of treatment | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit No. | Initial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Comment |
| 4-32 | 7350 | 7000 | 8800 | 8550 | 6500 | 6750 | 4700 | 3900 | 6700 | 9600 | 4600 | 3600 | Died on day 11 |
| 4-38 | 8100 | 6150 | 8000 | 14,300 | 8950 | 9150 | 4000 | 6200 | 7200 | 5050 | 17,300 | 10,450 | Continued on benzene and sulfapyridine 8 additional days without leukopenia |
| 4-50 | 5500 | 6700 | 8400 | 6800 | 7900 | 13,500 | 6800 | 5400 | | | | | Died on day 8 |
| 5-01 | 13,900 | 13,800 | 11,300 | 13,200 | 15,500 | 13,600 | 15,700 | 11,700 | | | | | Sacrificed on day 7 for pathological examination |
| 5-02 | 11,900 | 12,400 | 14,300 | 17,600 | 8900 | 21,500 | 18,000 | 15,200 | 14,100 | 13,150 | 12,950 | 16,900 | Continued 5 more days without leukopenia |
| 5-03 | 11,650 | 8900 | 11,900 | 15,700 | 15,900 | 9600 | 6700 | 9500 | 6700 | | | | Died on day 8 |
| 5-04 | 16,300 | 11,400 | 9700 | 11,300 | 7300 | 8000 | 11,600 | 5300 | 3000 | | | | Died on day 8 |
| 5-05 | 8500 | 14,700 | 11,400 | 15,400 | 12,300 | 9200 | 17,200 | 17,800 | 16,000 | 15,050 | 9100 | 12,500 | Continued 3 more days without leukopenia |
| 5-06 | 11,400 | 17,700 | 12,800 | 12,400 | 10,600 | 9200 | 9400 | 7900 | 10,900 | 5650 | 8200 | 12,400 | Continued 7 more days. Moderate leukopenia on last day |
| 2-37 | 11,800 | 10,500 | 14,200 | 10,500 | 7600 | 11,600 | 7400 | 9100 | 5000 | 3800 | 6600 | 6700 | |
| 2-05 | 14,000 | 18,200 | 19,800 | 12,800 | 13,200 | 15,100 | 11,300 | 10,500 | 10,300 | 22,000 | 14,400 | 13,300 | |
| 2-07 | 3800 | 10,500 | 8900 | 12,300 | 11,800 | | | | | | | | Died on day 5 |

The detailed data, recorded in Table I, give the daily counts of these rabbits for the first 12 days. Only 6 of the 123 counts recorded fall below 5000 cells per c. mm. The lowest leukocyte count in any of the animals was 3000 (rabbit 5-04) occurring on the day of death, which was 8 days after the beginning of benzene and sulfapyridine administration. One other animal of this group (rabbit 4-32) died with a moderate leukopenia, 3600 cells per c. mm., after 11 days. The count of 5400 occurring in rabbit 4-50 at the time of death on the 8th day was the same as the initial count of 5500.

Differential leukocyte counts were made at 3 to 4 day intervals on seven of the animals of this group. In general, the variations observed were not greater

than those that occur in normal animals. The percentage of polymorphonuclear leukocytes increased moderately in three of the animals, decreased in two, and remained essentially unchanged in two. Immature cells of the granulocytic series, other than metamyelocytes, were not found on the smears.

Concerning the inactivating effect of sulfapyridine on the leukotoxic action of benzene, it is interesting to note that other toxic manifestations of benzene poisoning did not appear to be entirely eliminated. Anorexia, loss of weight, extreme weakness, and diarrhea occurred in varying degrees of severity in many animals of the control group and also members of the sulfapyridine and benzene group which maintained normal counts. Even death occurred in four of the animals receiving benzene and sulfapyridine (rabbits 4-32, 4-50, 5-03, 2-07) although the leukocyte count was normal in two and the leukopenia was not unusually severe in the other two. However, since only four of the animals of this group died while under observation whereas all of the animals receiving continued injections of benzene alone died within 12 days, it appears likely that sulfapyridine served as an antidote for the general intoxication but to a considerably less degree than for the leukotoxic effect of benzene.

Attempts were made to determine how long the beginning of sulfapyridine therapy might be delayed after starting benzene injections and still obtain the antileukopenic effect. In four animals sulfapyridine was first given only after the leukocyte count had been reduced to about 3000 cells per c. mm. In three of the rabbits definite inhibition of the continued development of leukopenia was indicated by the fact that subsequent counts either failed to decrease progressively or actually increased. In two animals which were started on sulfapyridine when the count had reached 1000 per c. mm., the leukopenia followed an unmodified course and death occurred on the 2nd day after beginning sulfapyridine. Although these experiments are not sufficiently complete to justify final conclusions, they suggest that the effectiveness of sulfapyridine in counteracting the toxicity of benzene is determined by the extent of the damage produced by the benzene prior to the beginning of sulfapyridine therapy.

*Group 3. Effects of Sulfapyridine Alone.*—In a third group of animals sulfapyridine was given without benzene. Seven rabbits received 1.25 gm. *per os* twice daily for periods of 11 to 25 days to determine the effect of the drug on the number of circulating leukocytes. Interest was directed toward the possibility that sulfapyridine might effect stimulation of the marrow, as reflected by leukocytosis, which might in itself antagonize the action of benzene. That such a possibility warrants consideration is indicated by the studies of Weiskotten and Steensland (12) who reported the failure of benzene to produce leukopenia in four rabbits suffering from spontaneous acute infections. The investigators observed that three of the four rabbits developed leukocytosis and in all of them the percentage of polymorphonuclear leukocytes increased

under benzene treatment. The failure of benzene to produce leukopenia under these conditions suggested to the authors that the marrow of rabbits, under the stimulating leukopoietic effect of infection, is able to withstand the toxicity of benzene or is more resistant to its action.

A curve of the average leukocyte count of the seven animals receiving sulfapyridine alone is included in Chart 1. As in the case of the animals receiving sulfapyridine and benzene, the daily fluctuations of the count in this group of animals were greater than those observed in normal animals. However, in the composite average plotted in the chart the counts of a single animal were responsible for a large part of the wide variations. This animal is the only one of the seven in which the response of the leukocyte count gave evidence of a stimulating effect, and even in this instance, the count fluctuated irregularly between 12,000 and 31,600. The leukocyte count of the remaining six animals tended to show a downward trend which was most striking in one animal in which a count of 3800 was reached after 9 days and in another in which the count was 2600 after 16 days. Differential leukocyte counts in this group of rabbits revealed no increase in the percentage of granulocytes during the period of administration of sulfapyridine.

Because of the relatively wide fluctuations observed in the group of rabbits receiving sulfapyridine alone, as well as in those receiving sulfapyridine plus benzene, it seems not unlikely that sulfapyridine exerts an effect on the leukopoietic system. The nature of the reaction has not been studied. However, the findings do not warrant the explanation of the antileukopenic action of sulfapyridine as being referable to an overcompensating leukocytosis.

*Group 4. Effects of Benzene and p-Aminobenzoic Acid.*—It has been demonstrated by Woods (13) that *p*-aminobenzoic acid inhibits the bacteriostatic effect *in vitro* of sulfanilamide. Selbie (14) found that it also nullified the action of sulfanilamide in experimental streptococcal infections in mice, and our results (15) indicated a similar inhibitory effect on the action of sulfapyridine in experimental pneumococcal infections of mice. Woods (13) advanced the theory that *p*-aminobenzoic acid is essential for the growth of the organism and that the sulfonamide drugs interfere with its utilization by virtue of their structural relationship to *p*-aminobenzoic acid.

In view of the similarity in chemical structure between *p*-aminobenzoic acid and sulfapyridine and the antagonistic relationship between the two drugs in the bacterial studies, six animals were given *p*-aminobenzoic acid coincidentally with benzene as an additional control on the relative specificity of sulfapyridine. Three of them received 1.0 gm. twice daily and three 0.5 gm. twice daily. Mol for mol, the 1.0 gm. dose was larger than the dose of sulfapyridine used and the 0.5 gm. dose was smaller. The fall in the leukocyte count of the six rabbits was in no way different from that observed in rabbits receiving benzene alone. The curve of the average counts given in Chart 1 reveals how closely the response of this group paralleled that of the group receiving benzene alone.

An attempt was made to determine whether *p*-aminobenzoic acid, in accordance with its inhibitory effect on the antibacterial action of sulfapyridine, would influence the effect of sulfapyridine on the response of the leukocytes to the injection of benzene. For this purpose three rabbits received sulfapyridine plus *p*-aminobenzoic acid plus benzene. The experiment failed of conclusive results, because all three animals which received the three substances simultaneously became ill very rapidly and died within 3 to 5 days. The observations of James (16) on the depletion of acetate precursors as a cause of toxicity from sulfanilamide and sulfapyridine may be pertinent to the acute toxicity displayed by these animals.

*Group 5. Normal Rabbits.*—Although there are many reports in the literature on the course of the leukocyte counts of normal rabbits, daily estimations of the white blood cells were made on eleven normal rabbits as an additional control in the present study. When these rabbits are considered as a group and attention is directed to the average of the leukocyte counts of the 11 animals (Chart 1), it will be observed that the daily variations are small. The range between the highest and lowest average counts is less than 1000 cells, and therefore the average counts during the period of observation did not show variations of more than 9 per cent.

*Chart 1.*—In Chart 1 all of the results which have been separately described are consolidated. The salient features are as follows:

1. The rapid, progressive fall of the leukocyte count in the group receiving benzene alone and the group receiving benzene plus *p*-aminobenzoic acid.
2. The absence of a significant fall in the curve of the average leukocyte count of the group receiving benzene plus sulfapyridine.
3. The lack of evidence of sustained leukocytosis in the curve of the animals receiving sulfapyridine alone.
4. The wide daily fluctuations in the average leukocyte count of the group receiving sulfapyridine plus benzene as well as the group receiving sulfapyridine alone when compared with those of a group of normal rabbits.

*Chart 2.*—In Chart 2 are recorded the leukocyte counts of one animal of the benzene group and one animal of the sulfapyridine plus benzene group which were sacrificed for pathological examination on the day of the eighth injection. The chart makes evident the rapidly developing leukopenia of the rabbit on benzene alone and the maintenance of normal counts, ranging between 11,300 and 15,700, in the rabbit receiving benzene plus sulfapyridine. Photomicrographs of the femoral marrow of the two rabbits, along with the marrow of a normal, untreated rabbit, are presented in Figs. 1 to 3. The sections of marrow are from corresponding areas, and in each case are representative of the condition of the marrow in all areas sampled. They serve primarily to illustrate the influence of sulfapyridine in preventing the extreme aplasia which consistently results from injections of benzene in rabbits. The marrow of the animal which received sulfapyridine concurrently with the benzene shows an

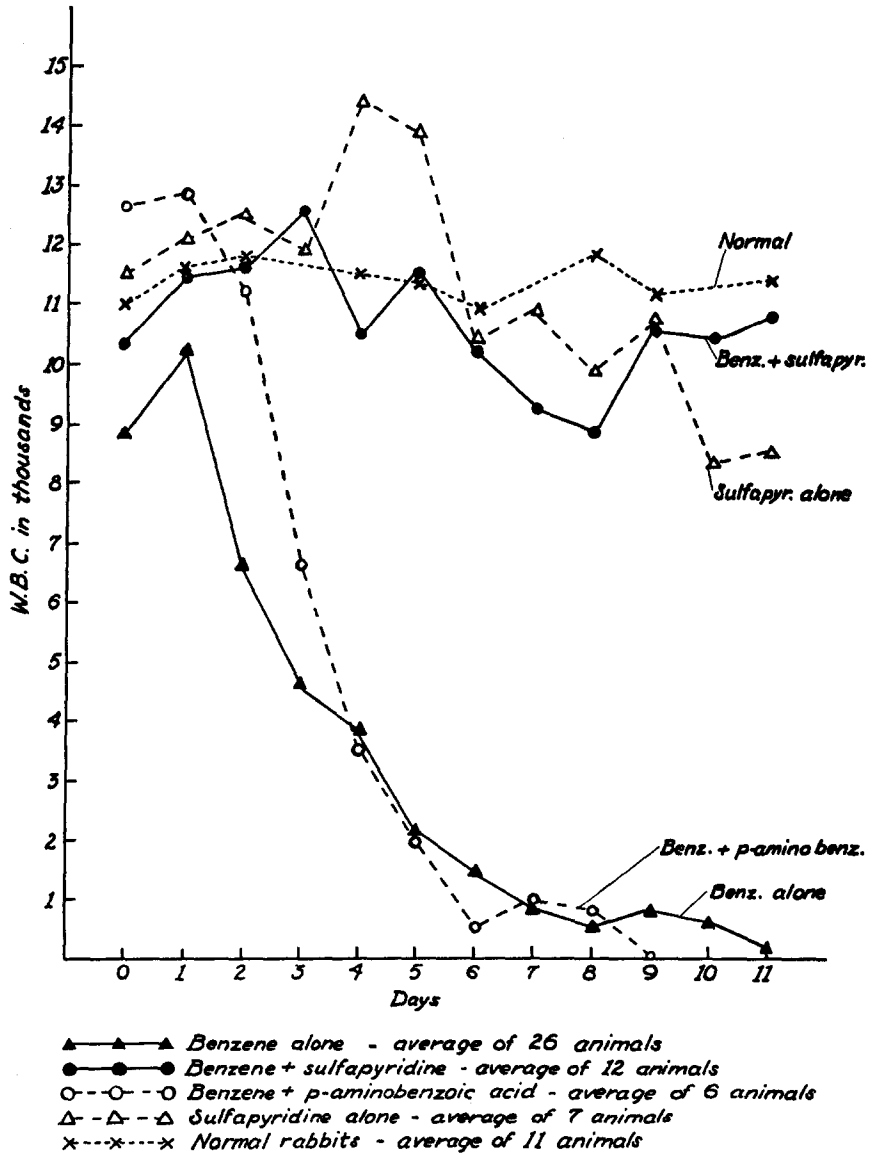


CHART 1. Curves of the composite average of the daily leukocyte counts of all the rabbits in each group.

abundance of hemopoietic tissue. Furthermore, some of the megakaryocytes have hyperchromic and pyknotic nuclei. The latter finding differs from the usual appearance of the marrow often found in untreated rabbits. In spite of

the wide variations which may exist in normal rabbits, the morphological evidence of "irritation" or "stimulation" suggests the special effect induced by sulfapyridine alone or in combination with some of products of benzene. A detailed analysis of the reaction of hemopoietic tissue to the chemical reagents requires qualitative studies not contained in the scope of this report. How-

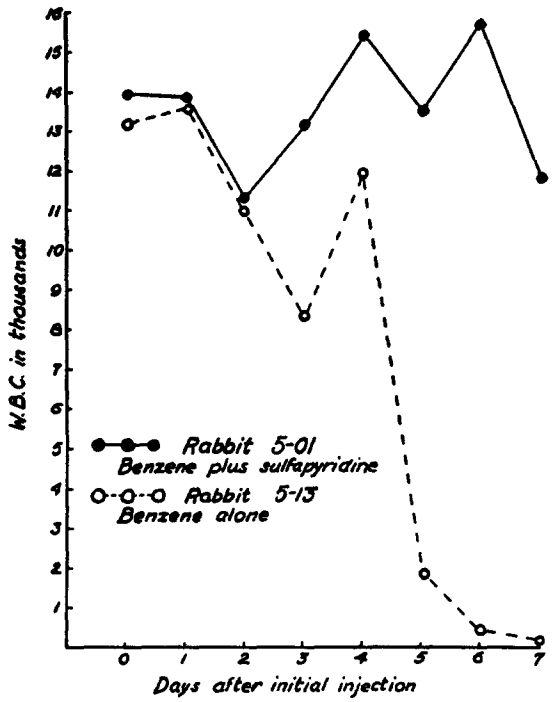


CHART 2. Daily leukocyte counts of rabbit 5-01 (benzene and sulfapyridine) and rabbit 5-13 (benzene alone) which were sacrificed for pathological examination. See Figs. 1 and 2.

ever, the comparable sections of bone marrow from the experimental animals reflect the quantitative results noted in the leukocytes of the circulating blood.

*Urinary Excretion of Phenols.*—As stated earlier, benzene injected into the animal body has been reported to be oxidized in part to phenols, (1). That one or more of the phenolic oxidation products may be responsible for the leukotoxic effect of the injected benzene has been suggested, (2, 3). In accordance with this possibility, the urinary phenols were studied in a small group of animals to determine whether the administration of sulfapyridine quantitatively influenced the excretion of phenols following subcutaneous in-

jections of benzene. The animals were kept constantly in metabolism cages which allowed the collection of 24 hour urine specimens, and benzene and sulfapyridine were administered as in the preceding experiments.

In the course of a series of studies on benzene leukopenia, Brewer and Weiskotten (17) made estimations of the urinary phenols of two rabbits. They observed fivefold increases in the output of phenols after injection of a benzene-olive oil mixture. Our results in three rabbits receiving benzene alone are in accord with their findings. Normally, the untreated rabbits excreted 90 to 140 mg. of phenols in 24 hours. The excretion doubled, however, in the 24 hour period after the first injection of benzene and continued to increase after subsequent injections. One animal excreted 590 mg. of phenols in the second 24 hours, and the other two excreted maximum amounts of 575 mg. and 658 mg. on the 4th day.

When sulfapyridine was given to three animals in conjunction with benzene, the excretion of urinary phenols differed from that described in the animals receiving benzene alone. In each of the three the increase in output of total phenols was substantially less than in the rabbits receiving benzene alone. Furthermore, in two of this group there was in addition a decided increase in the percentage of conjugated phenols. For example, one animal with an excretion of 75 to 136 mg. of total phenols in 24 hours under normal conditions, showed no increase in the first 2 days after beginning the administration of benzene and sulfapyridine. Furthermore, the 24 hour values for total phenols did not exceed 200 mg. during 5 days of continued treatment. Another animal showed no increased excretion in the first 24 hours. When, however, in the second 24 hours the total phenols were doubled, 50 per cent of the total was present in the conjugated form.

The record of a single animal in which a study of the urinary phenols was made during periods of the administration of benzene alone, sulfapyridine alone, and benzene plus sulfapyridine, serves to illustrate the results obtained. Chart 3 presents in graphic form the data on the excretion of urinary phenols of this rabbit.

It will be noted that sulfapyridine alone when given over a 3 day period exerted no appreciable effect on the phenol excretion. Daily injections of benzene caused a marked increase in the urinary phenols and a maximum of 575 mg. was attained in the 24 hours following the fourth injection. In contrast with the results using benzene alone, during a 3 day period in which sulfapyridine was administered coincidentally with benzene, the increase in total phenols was slight and there was a definite relative increase in the fraction of conjugated phenols. The delayed rise which occurred on the second day after discontinuing treatment may be interpreted as being dependent upon the slow absorption of the benzene-oil mixture.

Although the number of observations on the urinary excretion of phenols is too few to justify detailed conclusions, the differences in the phenolic output of rabbits receiving sulfapyridine plus benzene as contrasted with the animals injected with benzene alone have been constant. Concerning the mechanism of the effect of sulfapyridine on the phenol excretion, several possibilities warrant consideration. On the assumption that the limited increase in the excretion of phenols, when benzene was injected into rabbits receiving sulfapyridine, reflects an actual decrease in the formation of phenols from benzene,

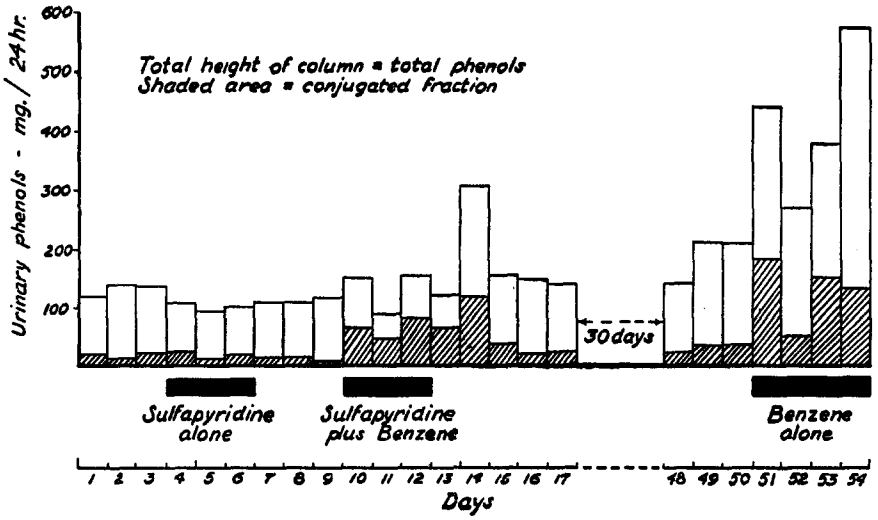


CHART 3. Urinary excretion of total and conjugated phenols of rabbit 2-12 during periods of treatment with sulfapyridine alone, sulfapyridine plus benzene, and benzene alone.

it seems possible that the presence of sulfapyridine in the tissues may influence the oxidation of benzene. Concerning the possible effect of sulfapyridine through the oxidative processes it may also be noted that benzene may be oxidized in the body to end-products other than phenols. Jaffe (18) has reported that disruption of the benzene ring resulting in the formation of the straight-chained muconic acid occurs in addition to the oxidation to phenols. On theoretical grounds, therefore, sulfapyridine may favor the processes involved in the breakdown of the ring and thereby reduce the amount of phenols or increase the formation of other, as yet undefined, end-products. Further study is required to appraise the validity of these possibilities. However, since the introduction of sulfapyridine is associated with the inhibition of the development of leukopenia and also brings about a reduction in the excretion of

free phenols which follows injections of benzene, the findings lend support to the possibility that phenolic substances or other end-products may be responsible for the leukotoxic action of benzene.

## SUMMARY

1. In rabbits receiving subcutaneous injections of benzene, the simultaneous administration of sulfapyridine, *per os*, prevented the development of leukopenia. The sparing effect of sulfapyridine on the intoxication of the leukopoietic tissue by benzene was demonstrated not only by the range of daily leukocyte counts but also by microscopic examination of the bone marrow of treated animals.

2. The administration of para-aminobenzoic acid failed to inhibit the leukotoxic action of benzene.

3. The administration of sulfapyridine alone to rabbits was followed by daily variation in the number of leukocytes in the circulating blood but persistent leukocytosis was not observed. The inhibiting action of sulfapyridine was not found to be referable to the development of an overcompensating leukocytosis.

4. In preliminary experiments, the excretion of phenols by rabbits receiving sulfapyridine together with benzene differed from that observed in animals receiving benzene alone. In the former group, the rise in the excretion of total phenols was not so high as in the latter group, but the percentage of combined phenols was greater. The possible significance of the findings has been discussed.

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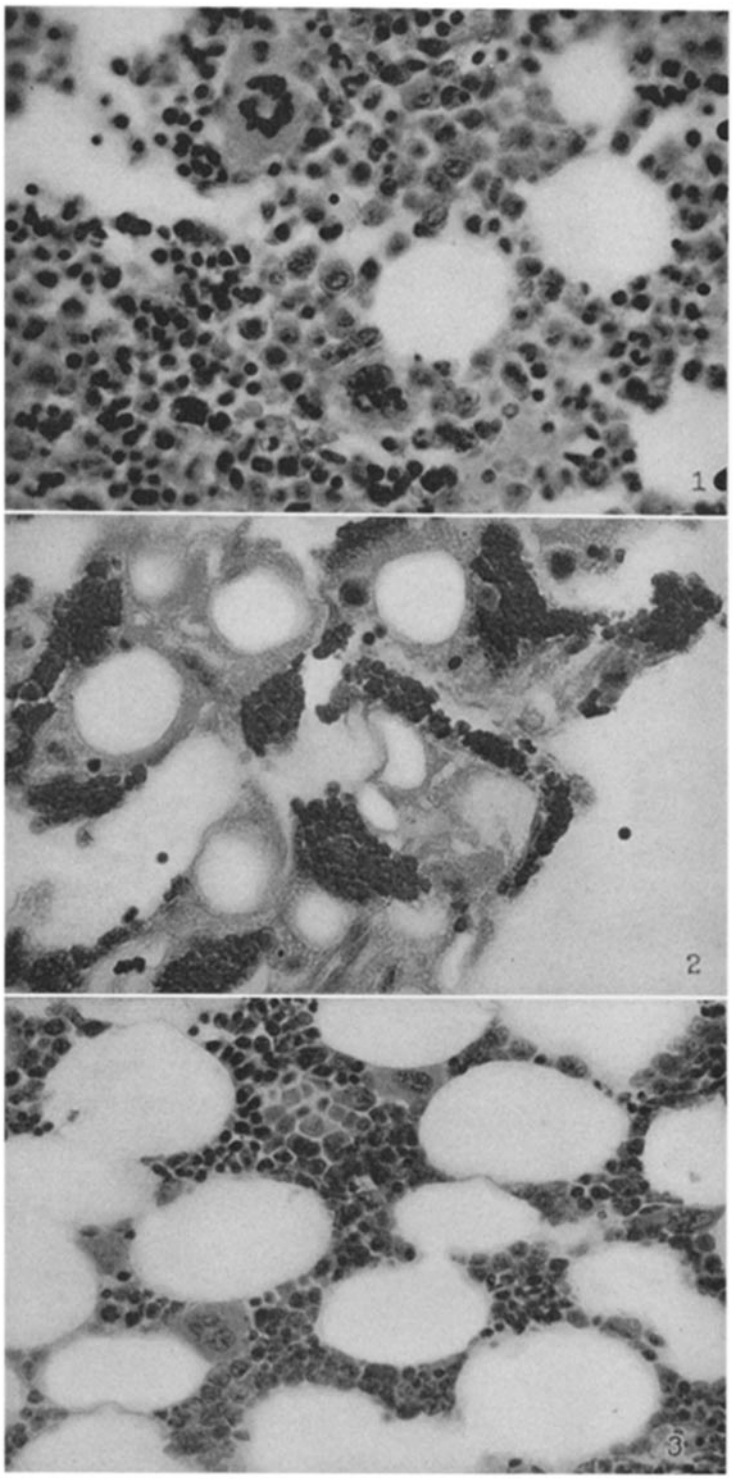
## EXPLANATION OF PLATE 24

We are indebted to Dr. Irving Graef, Department of Pathology, New York University College of Medicine, for the description of the sections appearing in Figs. 1 to 3.

FIG. 1. *Rabbit 5-01* (combined benzene and sulfapyridine): Sections of the femur marrow (sections of rib and vertebra are similar) reveal fatty marrow with large islands of hemopoietic tissue, normal types of cells and many young eosinophilic leukocytes. No "toxic" or degenerative forms were observed. Some megacaryocytes reveal hyperchromic and pyknotic nuclei but many are quite normal.

FIG. 2. *Rabbit 5-13* (benzene): Sections of the femur marrow (rib and vertebra are identical) reveal complete depletion of hemopoietic cells, congested and dilated marrow sinusoids and large fatty lobules. The nuclei of the fat cells have disappeared, as well as the hemopoietic cells.

FIG. 3. *Normal Rabbit:* Sections of the femur marrow (illustrated by the accompanying photograph) reveal a mixture of fatty tissue, islands of hemopoietic cells and marrow sinusoids. The pattern is fairly uniform, the cells are distributed in moderate numbers and exhibit the usual normal types.




(McCarty and Tillett: Sulfapyridine and leukotoxic action of benzene)