CORRELATION BETWEEN THE MEAN CORPUSCULAR VOLUME AND RETICULOCYTOSIS IN PHENYLHYDRAZINE ANEMIA IN SWINE

By F. DOUGLAS LAWRASON, Lt. (jg), MCR, USNR, D. C. ELTZHOLTZ, CPHM, USN, C. R. SIPE, CPHM, USN, AND P. K. SCHORK, CPHM, USN

ONE OF THE causes of an increase in mean corpuscular size is a pronounced reticulocytosis. The increase in the volume of the red blood cells due to this cause is usually a temporary finding which follows a sudden loss of blood, a hemolytic crisis, or any reaction which acutely stimulates the hematopoietic system. During treatment of pernicious anemia with specific therapy, it is not unusual to find in conjunction with the reticulocyte response a transient increase in the degree of macrocytosis as measured by the mean corpuscular volume.

When a macrocytic blood picture is associated with a reticulocytosis, it is often difficult to evaluate to what extent the larger size of the reticulocyte contributes to the mean corpuscular volume. This problem was encountered in the interpretation of hematologic data gathered during previous studies with swine at the Naval Medical Research Institute, Bethesda, Maryland. The present investigation was undertaken to study the correlation between the increased percentage of reticulocytes produced by phenylhydrazine hydrochloride and the mean corpuscular volume in swine under controlled conditions.

Phenylhydrazine has been used by numerous workers to produce both experimental anemia and reticulocytosis. This drug and its derivatives have been considered hemolytic agents. However, Goodman and Gilman do not consider the chemical action of the drug hemolytic in nature. They believe the drug enters the red cell, splits part of the hemoglobin to hemin and denatured globin; and the hemin, acting as a catalyst, changes the remaining hemoglobin to methemoglobin and possibly other unidentified substances. Phenylhydrazine usually does not cause depression of the bone marrow and probably does not affect the immature red cell or the white cell. Erythroid and myeloid hyperplasia have been noted in bone marrow of animals treated with the drug.

MATERIALS AND METHODS

Six adult swine averaging 185 pounds in weight were used for these studies. They were procured from a hybrid stock predominantly Duroc-Jersey with an admixture of Poland-China and Chester-White. All six swine were kept in a common pen measuring approximately 10 by 20 feet. Their diet, as recommended by the U. S. Department of Agriculture, consisted of a 17 per cent protein-vitamin-mineral mixture and 83 per cent whole yellow corn. Brucella abortus agglutination tests were negative.

Animals were bled in the fasting state. The blood was obtained from the deeply lying jugular veins in the lower neck. The bone marrow was aspirated from the sternum with a Turkel needle, and approximately 0.1 to 0.2 cc. of marrow blood was withdrawn. The preparation of the smear-imprint was made immediately upon aspiration. All other laboratory determinations were carried out by standard methods.

From the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.

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The same amount of phenylhydrazine hydrochloride was given to all of the animals on each day of treatment. The drug was given orally with a handful of food for the first nine doses. Beginning with the tenth dose and continuing to the end of the study, it was given intravenously in a 2 per cent aqueous solution into the same plexus of veins from which the animal was bled.

Results

Initially all 6 swine were considered hematologically normal with an average erythrocyte count of 6.9 million per cu. mm. (fig. 1). The average mean corpuscular volume (MCV) of the red cells for the group was 60 cubic microns and the reticulocyte count was 0.3 per cent. Phenylhydrazine hydrochloride was fed by mouth in daily doses of 0.1 to 0.4 Gm. for the first nine days. The dose was increased to 0.5 Gm. on the tenth day and was given intravenously (fig. 1). Little or no immediate reaction was noted with the administration of 0.5 Gm.; however, when 1.0 Gm. was given intravenously, the animals exhibited mild to moderate weakness following injection. This reaction lasted from one to three minutes. On the forty-second dose the drug was increased to 1.0 Gm. and the weakness following the injection became more severe and lasted five to ten minutes. The severe response observed with the higher dose may have been due to the fact that when it was administered, all animals were severely anemic, listless, and weak. On the fiftieth day the drug was discontinued.
One animal, number 5, failed to respond with a reticulocytosis. It became rapidly leukopenic and severely anemic and died from intercurrent infection on the fourteenth day, after receiving only 4.4 Gm. of phenylhydrazine. At autopsy the bone marrow exhibited an extreme hypoplasia of both erythroid and myeloid components. Since the response of this pig diverged widely from the rest of the group and from the usual response to this drug, the data gathered from this animal were not included with the data of the group.

The total dose given to the 5 remaining animals ranged from 0.30 to 0.35 Gm. per kilogram of body weight, or approximately 28 Gm. Of these 5 pigs, 3 died between the thirty-ninth and fiftieth day and the remaining 2 survived and recovered. The total dose received by each of the 2 surviving animals was 30.7 Gm. One animal was found to be pregnant toward the end of the experiment. Incomplete abortion occurred two days before death of the animal. During the last ten
FIG. 3.—Photomicrographs of bone marrow specimens obtained before and during the administration of phenylhydrazine HCl. (a) Bone marrow from one of the swine showing erythroid hyperplasia and multinucleated erythroblasts observed during the period of pronounced reticuloysisis and anemia. (b) Bone marrow showing numerous very immature erythroblasts during the period of hyperplasia. (c) Erythroid hyperplasia exhibiting many basophilic normoblasts. (d) Erythroid hyperplasia exhibiting late normoblasts. (e) Normal bone marrow obtained from one of the swine before the administration of phenylhydrazine HCl.
days of the study all of the animals developed sterile abscesses at the sites of injection.

Figure 1 demonstrates the course of the average red blood count (RBC) for the group of 5 swine. The RBC of 2 of the animals dropped below one million shortly before death but the lowest average level for the entire group was 1.6 million. It will be noted that the average reticulocyte count for the 5 animals was maintained above 50 per cent for one month, and over 90 per cent for sixteen days. During ten days of this latter period, 3 of the animals maintained a reticulocytosis of approximately 100 per cent. The hemoglobin and hematocrit decreased proportionately to the RBC but are not shown on the graph. At the time when the reticulocytosis was marked and when numerous Heinz-Ehrlich bodies were seen, the spectrophotometric determinations of the hemoglobin may have given falsely high readings because of the peculiar turbidity of the solution. A similar turbidity has been described for in vivo and in vitro studies with phenylhydrazine and has been considered to be due to the release of the Heinz-Ehrlich bodies from the erythrocytes.31

The average MCV closely followed the trend of the reticulocytosis and both reached a maximum simultaneously. The maximum MCV of the group average was 133 cubic microns (fig. 1). The MCV of one of the surviving animals which had maintained a reticulocytosis close to 100 per cent for ten days remained at 140 cubic microns during this period. In figure 2 the MCV is plotted with relation to the reticulocyte per cent for all determinations made in the five swine during the study. The regression line for this correlation is a straight line fitted to the data by the method of least squares. In determining this regression line, only the data up to 80 per cent reticulocytes were included. It can be seen that with each increment of 10 per cent in the reticulocytes the MCV increases 6.8 cubic microns plus or minus a standard deviation of 7.8 cubic microns. The reason for using the selected data will be discussed.

The bone marrow of all animals was studied periodically. Beginning at the eleventh to twentieth day and continuing throughout the time of administration of the drug, the bone marrows of the 5 swine exhibited marked erythrocytic hyperplasia (figs. 3a, 3b, 3c, 3d). The myeloid-erythroid ratio was reversed. From 25 to 75 erythroblasts were encountered for every immature white cell. Many of the erythroblasts were quite immature and commonly found in large aggregates containing many pronormoblasts and basophilic normoblasts. Frequent multinucleated erythroblasts were seen (fig. 3a). Mitoses occurred in every stage of maturation and unusual numbers were seen occurring in large "nests" of erythroid regeneration. A normal pig bone marrow is shown for comparison in figure 3e. The myeloid series did not appear to be disturbed. The peripheral leukocyte counts were erratic throughout the experiment but at no time did they reach leukopenic levels in any of the five animals. Most of the swine developed a leukocytosis terminally which was probably, for the most part, a reaction to terminal infection.

DISCUSSION

Clinically, a delayed reaction to phenylhydrazine, manifested by a progressive anemia sometimes occurring many days after discontinuation of the drug, is well
known. Experimentally, the effect of the drug upon the red cells seems to occur with little delay. Upon discontinuance of the drug, recovery from the anemia promptly occurs. In the 2 surviving swine no delayed effect of the drug on the RBC was observed.

There is some uncertainty as to whether the action of phenylhydrazine is hemolytic in character or is due to the aplastic effect of the benzol ring as some investigators believe. At any rate, animal number 5 reacted as if it were poisoned with benzol. The pig developed a marked leukopenia and progressive anemia within the first week and died on the fourteenth day of treatment. At autopsy an extensive hypoplasia of all elements in the bone marrow was found. The other 5 swine exhibited the usual response to phenylhydrazine. The variation in response observed in the one animal can not be explained.

The much discussed Heinz-Ehrlich bodies were seen in peripheral blood and for the most part appeared to be within the adult erythrocytes. Cruz considers this fact, among other evidence, to support the theory that phenylhydrazine attacks only the adult and not the immature cell. He believes that these refractile bodies are evidence of destruction within the red cell. No observations were made in this study on whether or not phenylhydrazine attacks only the adult erythrocyte. However, in view of the extreme erythroid hyperplasia in the bone marrow and the high reticulocytosis in the peripheral blood during intravenous administration of the drug, it would appear that the drug did not attack the immature red cell.

The data used for the calculation of the regression line seen in figure 2 were those occurring below the 80 per cent reticulocyte level. Above this level a more extensive scattering of points and apparent lack of continued close linear correlation occurred. During this period of observation the animals were extremely ill, severely anemic, and 3 of the 5 died. The 2 surviving pigs appeared moribund when the drug was discontinued. Wintrobe has pointed out that in pernicious anemia a close correlation exists between the erythrocyte count and the MCV of the red cells when the anemia is moderate, but when the anemia is extreme a close correlation is not found. Similarly, in these swine, the correlation between the number of reticulocytes and the MCV probably was affected by the severity of the anemia.

Qualitatively the bone marrow during this period did not appear to be as hyperplastic as it did earlier in the experiment. Even though the majority of the red cells in the peripheral blood were reticulocytes, it does not seem likely that the erythroid regeneration in the bone marrow could have been proceeding at an ideal maximum rate since the general metabolism was undoubtedly severely disturbed. It may be that at the higher dose of phenylhydrazine practically all of the adult erythrocytes were destroyed thus leaving only reticulocytes in the peripheral blood. Therefore, at the near 100 per cent level, the reticulocyte response is probably only an apparent maximum and not a true index of optimum erythrocytic regeneration.

However, in spite of the wide scattering of values between the 80 and 100 per cent reticulocyte level, it appears that the majority of the points tend to cluster toward MCV values lower than expected. The reason for this is not entirely clear. If the animals were iron deficient during this period of observation, the smaller mean corpuscular volume of the red cells may possibly be explained. Experimental
anemia produced by phenylhydrazine usually is not considered to be complicated by an iron deficiency since the iron from the destroyed cells is returned to the system for new hemoglobin formation. However, sterile abscesses developed at the site of injection of the drug in all of the swine. Robscheit-Robbins and Whipple and others have shown that in the presence of a chronic inflammatory reaction, such as a sterile abscess, the rate of production of new hemoglobin diminishes because iron is diverted to the tissues and is not made available for hemoglobin synthesis. Therefore, toward the end of the experiment the anemia may have become an iron deficiency anemia.

Another explanation is based upon the previously discussed possibility that the bone marrow was less active during the period when the majority of circulating cells were reticulocytes. Thus, the relative percentage of nearly mature, and therefore smaller, reticulocytes would increase. This trend, if pronounced, may have become sufficient to account in part for the apparent shift of the previously linear correlation. In the final analysis, it is likely that many factors affected the correlation in this high range of reticulocytosis.

Below a reticulocytosis of 80 per cent, a close correlation between the per cent of reticulocytes and the mean corpuscular volume was found. However, since there is a large variation in individual determinations as evidenced by the standard deviation of 7.8 cubic microns, it would be difficult to attribute a macrocytosis to an associated reticulocytosis if the reticulocytes were not increased beyond 10 per cent. Nevertheless, from the data it is possible to determine approximately the role a moderate reticulocytosis would play in the increased mean corpuscular volume found in a macrocytic blood picture in swine.

SUMMARY AND CONCLUSIONS

1. Six adult swine were given phenylhydrazine hydrochloride orally and intravenously. Hematologic observations, which included periodic bone marrow studies were made before, during, and after the administration of the drug.

2. Five swine responded to the drug in the usual manner with progressive anemia, reticulocytosis, and erythrocytic hyperplasia of the bone marrow. Three animals died between the thirty-ninth and fiftieth day of the experiment after receiving a total dose of 0.30 to 0.35 Gm. per kilogram of body weight. Two swine survived and recovered after receiving a similar dose.

3. One animal died on the fourteenth day of the experiment and exhibited a course which closely resembled that of benzo poisoning. Rapid and progressive granulocytopenia, anemia, and extreme universal hypoplasia of the bone marrow were observed.

4. A direct correlation between the mean corpuscular volume of the red cell and the per cent reticulocytes was found within the limits of 0 to 80 per cent reticulocytosis. With each increment of 10 per cent in the reticulocytes the mean corpuscular volume increased approximately 6.8 cubic microns.

REFERENCES


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