INCIDENTAL FINDING OF MEGALOBLASTIC-LIKE CELLS IN BONE MARROW OF ONE OF TWO SWINE WITH MACROCYTIC ANEMIA AND ACHLORHYDRIA*†

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Introduction

Ever since Ehrlich described the megaloblasts seen in pernicious anemia during relapse, there have been attempts by many investigators to produce experimentally an anemia similar in all respects to human pernicious anemia. Macrocytic anemia with the megaloblastic type of erythrocytic regeneration in the bone marrow, achlorhydria, and a specific response to a substance containing the anti-anemic principle are the criteria necessary to establish the presence of such an anemia.

Thus far, efforts to produce a macrocytic anemia in experimental animals have not been uniformly successful, but it is agreed by many investigators that swine constitute a suitable experimental animal in which to study this problem.[3, 16, 25, 32] Total gastrectomy[1, 9, 15, 17, 18, 30] alone, or combined with liver damage,[23] has produced only an occasional macrocytic anemia in animals. The usual resultant anemia is normocytic and hypochromic and in most cases responds to iron therapy. Davis[4] reported the development of a "hyperchromic" anemia in dogs given daily doses of choline chloride. This not only responded to liver therapy but also to atropine sulfate, and on this basis the anemia was not considered to be due to a specific lack of the anti-anemia factor.

Of extreme interest are the recent reports of Welch,[25] Heinle,[10] Cartwright,[2] and their associates, who have been able to produce macrocytic anemias in swine fed diets deficient in pteroylglutamic acid and supplemented with a chemical antagonist of pteroylglutamic acid. The difference

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in the basic diets fed to the different groups of swine by the above investigators may be relevant in attempting to explain the varying response of the experimental anemias in these animals to treatment with extrinsic factor and normal human gastric juice, liver extract, and an increase in protein in the diet. However, the anemia of all the animals rendered deficient in pteroylglutamic acid did respond favorably to treatment with pteroylglutamic acid. Experimental macrocytic anemias can also be produced by feeding diets deficient in B complex vitamins but this method is not entirely satisfactory.[3, 16, 24, 25, 26, 30] Many of the anemias produced by these means were found to respond to a factor contained in yeast, related possibly to the vitamin B complex group, yet not identifiable with any one vitamin, and at times unresponsive to purified liver extracts.[17, 19, 27, 28, 32] In view of these experimental data and clinical evidence, Wintrobe[30] and others have raised the question whether or not pernicious anemia is in reality a multiple deficiency disorder rather than one caused by the lack of a single substance.

The identification of the megaloblast presents a cytological problem that has been the cause of much disagreement for many years. Jones,[11, 12, 13] Downey,[7, 8] Wintrobe,[31] and others believe that megaloblasts are present only in fetal bone marrow and in pernicious anemia or anemias in which the antipernicious anemia principle is lacking, and are not found in normal bone marrow. On the other hand, certain hematologists, principally Sabin[20, 21] and Doan,[5, 6] consider the megaloblast as an early stage in the development of the erythroblast and to be present in normal bone marrow. The main criticism of this latter observation is the technique of supravital staining upon which it is based. Jones contends that the delicate chromatin pattern of the nucleus, which is seen in the dry smear and lost in the supravital stain, is the most important criterion for the identification of the megaloblast. At the same time he finds it quite impossible to identify the megaloblast in imbedded tissue sections and would thus be unable to confirm their presence in the bone marrow of the experimental monkeys with macrocytic nutritional anemia as reported by Wills *et al.*,[26, 28] and in the swine with anemia as claimed by Miller and Rhoads.[16] Wintrobe[32] in 1939 reported the presence of cells which had the appearance of "megaloblasts" in the bone marrow of pigs with normocytic anemia due to a deficiency of water-soluble vitamins and other substances contained in yeast. He was unable to make a positive identification of those cells at that time. However, recently, two groups of investigators have reported the presence of cells resembling megaloblasts in the bone marrow of swine deficient in pteroylglutamic acid.[2, 25]

The following preliminary report is based on observations made in two swine during seventeen months following exposure to atom bomb ionizing radiation at Bikini. Both hogs were in the group of experimental animals placed aboard target ships to be studied for the effects of total body radiation from the air-burst bomb. Animal A received minimal amounts of ionizing radiation while animal B was exposed to considerable amounts of radiation.

Experimental background

In order to evaluate the hematologic changes found in the two swine during the seventeen months following exposure to ionizing radiation, it will be necessary to examine in some detail the background and the possible exciting causes which may have been involved in the development of the macrocytic anemia. Both animals were fifteen weeks of age at the time of exposure, 1 July 1946, and each had been observed for about six weeks prior to that time. They had been obtained from a single herd, predominantly of Duroc-Jersey stock, with an admixture of Chester-White and Poland-China stock. At that time agglutinations for Brucella were negative.* During the period aboard ship and continuing for eighty-five days following exposure, all swine were maintained on a diet somewhat restricted in calories. However, except for the restriction in total calories, the diet was considered nutritionally adequate for growing swine in respect to protein, vitamin, and mineral requirements. The swine gained an average of one pound a day in weight during the fifteen-day period before the detonation of the bomb. Animals A and B gained at this rate and continued to develop to the normal adult size weighing approximately 200 pounds each. The diet consisted of crushed oats 90%, meat scraps 10%, and a swine salt mixture 1%. Alfalfa hay was given *ad lib.* until the supply was exhausted, approximately ten days before the animals were transferred from the ship to the Naval Medical Research Institute, Bethesda, Maryland. During observation at the Institute, their diet was basically composed of 17% protein-mineral mixture and 83% whole yellow corn as recommended by the U.S. Department of Agriculture. A commercial vitamin-mineral mixture supplied the necessary vitamins and minerals. For the period of six months after arrival at the Research Institute, alfalfa meal was not available so the tankage was increased proportionately. The amount of the

* Agglutination tests were performed by the California State Department of Agriculture.

diet fed the animals once daily depended upon the amount consumed *ad lib.* with a portion being left in the feeding trough.

In addition to the above dietary changes, the following factors might also be considered as variables in the overall experiment involving swine at Bikini. Approximately ten days after the bomb burst the herd was found to be infected with *Ascaris suis*. Sodium fluoride, 1% by weight of a daily ration, was given once to each pig. This amounted to about 9 grams of sodium fluoride per animal, a dose considered sufficient to eradicate the infestation.

Another factor which may have contributed to a nutritional deficiency and may have altered the blood picture was chronic hog cholera. Aboard ship a few animals died; the clinical course and autopsy findings of these animals were similar to those of animals that have since died of proven atypical chronic hog cholera* at the Naval Medical Research Institute. These animals presented a profound picture of malnutrition. The incidence and the effect on hematopoiesis of chronic hog cholera in the herd aboard ship and after arrival at the Institute are unknown. Undoubtedly, a chronic infection such as chronic hog cholera would influence the hematologic profile of the affected swine. The influence of age on hematopoiesis is of importance and will be considered along with the "observations."

Observations

Figure 1 shows a comparison of the total RBC of the heavily irradiated swine, the biologically insignificantly irradiated swine aboard the target ships, the swine which were retained aboard the animal laboratory ship, the *USS Burleson,* and the two animals under consideration in this report. It will be noted that all control counts were between 5.5 and 6.0 million. Scarborough[22] reports that the normal range of the RBC in swine is between 5.0 and 9.0 million, with an average of 6.7 million. Likewise the control hemoglobin, hematocrit, and blood indexes were within the normal range. The RBC of both the heavily irradiated and insignificantly irradiated groups (*i.e.,* 90 animals aboard target ships that were beyond the range of significant radiation) decreased at about the same rate and followed the same trend. The RBC of the swine that never left the *Burleson* also were found to be as low as those of the test animals 30 days after the test. All three groups recovered at about the same rate, and the beginning of the recovery period coincided with the correction of the calorically restricted

* Proved by biologic test by the U.S. Department of Agriculture.

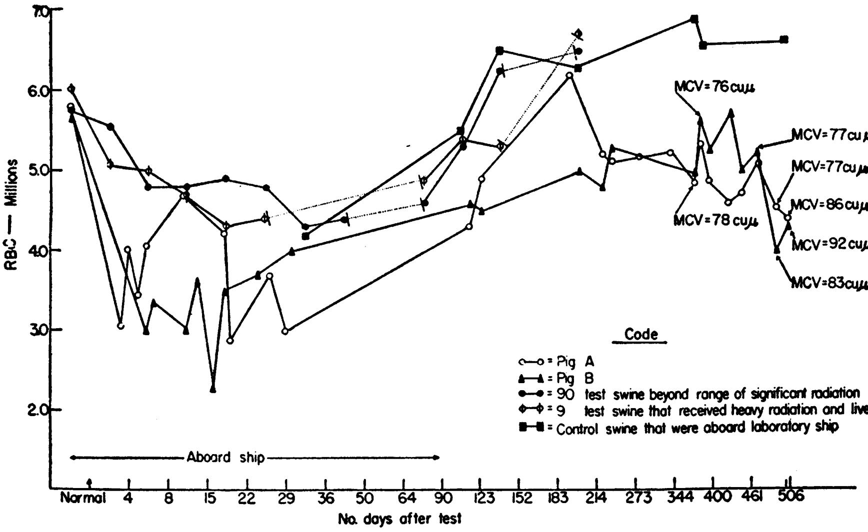

FIG. 1. Comparison of RBC of swine A and B with heavily irradiated and insignificantly irradiated swine exposed to atomic bomb air burst test.

diet. The final counts at the time of this study in the mature animals (608 days) ranged between 6.5 and 7.0 million RBC per cu. mm. Since Wintrobe,[29, 32] Smith,[24] and others have stressed the importance of nutrition, initial estrus, and other factors and their effect upon the hematologic status in young swine, it is realized that these factors probably played an important part in the overall blood picture and are possibly reflected in the trend of the RBC of the animals.

The course of the RBC in animals A and B can also be followed over this period of time. In regard to the RBC, however, neither of these animals completely recovered as the rest of the group did. The peripheral blood has continued to manifest moderate degrees of anisocytosis, poikilocytosis, and polychromasia, and in many respects these abnormalities have become more pronounced (Fig. 2). The volume of the red cells has been considerably greater than normal. The mean corpuscular volume (MCV) of animal A has been greater than 71 cubic microns in all determinations except one; the last determination at the time of this study was 86 cubic microns. Likewise, the MCV of pig B has become progressively greater; the last five determinations were 76, 74, 77, 83 and 92 cubic microns. The mean diameters of the RBC are increased as well in both animals. This is considered a true macrocytosis and not just a reflection of a reticulocytosis,[14] since on repeated determinations the reticulocyte count has ranged between 0.5 and 1.3%.

Animal A received a minimal amount of ionizing radiation, probably in the range of 50 roentgens, calculated from the dosage recorded by film in the same compartment aboard the target ship and from the biological effect. This latter effect is studied best at the present time by following the total WBC* and differential counts after exposure to radiation. Figure 3 graphically compares the total WBC in the same two groups and animals A and B throughout the same period of time. In the immediate post-irradiation

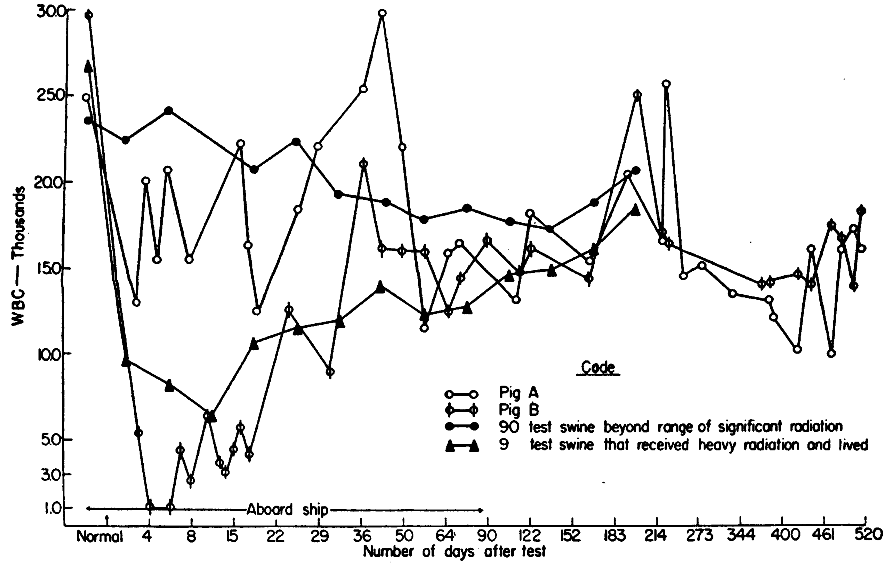

FIG. 3. Comparison of WBC of swine A and B with heavily irradiated and insignificantly irradiated swine exposed to atomic bomb air burst test.

period, pig A showed only a moderate drop, not of the same degree as the average leucocyte decrease of the nine more heavily irradiated animals that survived at least six months. The recovery of the WBC occurred gradually over three to five months.

The typical leucocyte response following irradiation of pigs is clearly outlined in the course of the WBC in animal B. Of the two pigs in the same

* Although the total lymphocyte count is generally considered as the best index to exposure to radiation, the statistical analysis of Mr. W. V. Charters (Statistical Division of the Bureau of Medicine and Surgery) has shown the total WBC of swine to be equally significant (unpublished).

compartment aboard the target ship with B, one was found dead on the first day and its death can not be satisfactorily explained. The second died on the fifth day with a generalized hemorrhagic diathesis. The photographic dosimeter in the compartment recorded about 3,500 roentgens of radiation. However, the fact that animal B is still alive constitutes evidence that it received only a fraction thereof. A possible explanation is that the animal may have been protected by heavy machinery or other shielding. The higher average WBC seen early in the experiment may have been due to immaturity of the entire group.

Repeated gastric analyses before and after histamine failed to reveal any free hydrochloric acid in either of these animals. However, Wintrobe[32] states that the achlorhydria found in his experimental swine was not persistent and varied with repeated determinations. Icteric index and Van den Bergh determinations have been within normal limits. Since it was not feasible to carry out serial bone marrow studies on all experimental animals, early bone marrow imprints are not available. Studies of the marrow were first performed on animals A and B approximately one year after exposure to the atom bomb and have been repeated at monthly intervals. The technique of repeated sternal aspiration and examination of the dry smear-imprint has been entirely satisfactory.

The bone marrow from animal A is moderately hyperplastic with a definite increase in the number of early erythrocytic cells. The myelo-erythrocytic ratio is 3 to 1. The majority of the immature erythroid elements manifest the usual pattern of the normoblastic series. Their nuclei are composed of relatively thick strands of chromatin which have the early tendency to clump, and as the cell matures, it assumes the "Radkern" type nucleus. These cells are of normal size and contain the normal nucleocytoplasmic ratio. However, there is a significant number of immature erythroid cells that markedly resemble the megaloblasts seen in the bone marrow of pernicious anemia in relapse (Fig. 4). The very early forms, the "pro-megaloblasts," are larger and contain more cytoplasm than the pro-normoblasts seen in the bone marrow of the control animals. The chromatin of the nucleus has a fine, reticulated pattern which is distributed uniformly throughout. The delicate strands are widely separated by parachromatin and fail to show any tendency to clump. One or two nucleoli are usually present. We have labeled the next distinct stage in maturation the "basophilic megaloblast"; these are comparable to the basophilic normoblast of normal erythropoiesis. These cells also are larger and contain more cytoplasm which is somewhat less densely basophilic than its counterpart in the normoblastic series. The chromatin pattern of the nucleus continues to show the delicate scroll design with little evidence of clumping. About 30 to 40% of the immature hemoglobiniferous cells at this stage are of the megaloblastic-like type.

As maturation proceeds, the nuclear distinction between the two series becomes less marked since there is also evidence of clumping of the "megaloblastic" nucleus. However, these near-mature "megaloblasts" continue to be considerably larger and in many cases are twice the size of the corresponding polychromatic or orthochromatic normoblast. Furthermore, the cell contains more cytoplasm and the nucleus remains larger. That these cells are the precursors of the macrocytes seen in the peripheral blood is apparent through the various intermediary stages in their maturation. Only an occasional, large neutrophilic meta-myelocyte, like those which have been described in the megaloblastic marrow of pernicious anemia, is seen. Multilobulation of the nucleus of the mature neutrophil in the peripheral blood is absent.

In comparing the typical megaloblasts found in pernicious anemia with the megaloblastic-like cells in animal A, differences can be noted. However, these differences are believed by the authors to be for the most part interspecies differences, since in the marrow of swine, these cells differ as markedly in their characteristics from the usual normoblastic pattern seen in the controls as the human megaloblast does from its normoblastic counterpart. It is believed that all of the cytological criteria for the identification of the megaloblast are present.

Animal B also manifests a macrocytic anemia with anisocytosis, poikilocytosis, and polychromasia. This animal definitely received more radiation than pig A, but from the hematologic standpoint they have followed a similar course over the past eight months. As yet no cells similar to megaloblasts have been found in the bone marrow of animal B even though a moderate degree of erythroid hyperplasia is present. It has been considered desirable to observe the spontaneous course of these two animals over a longer period of time; therefore, specific therapy has been withheld.

Comments

It is not the aim of this report to propose a relationship of cause and effect between any of the variables mentioned and the appearance of the "megaloblastic" macrocytic anemia. There is little possibility of complete evaluation of the important variables of nutrition, radiation, and disease that were present during the development of these swine. However, it

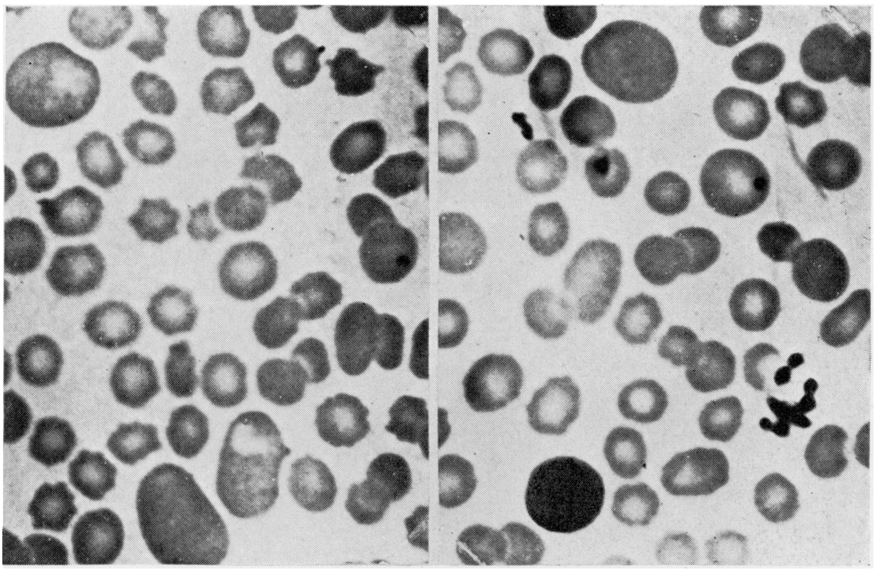

FIG. 2. Peripheral blood smears from pig A (left) and pig B (right).

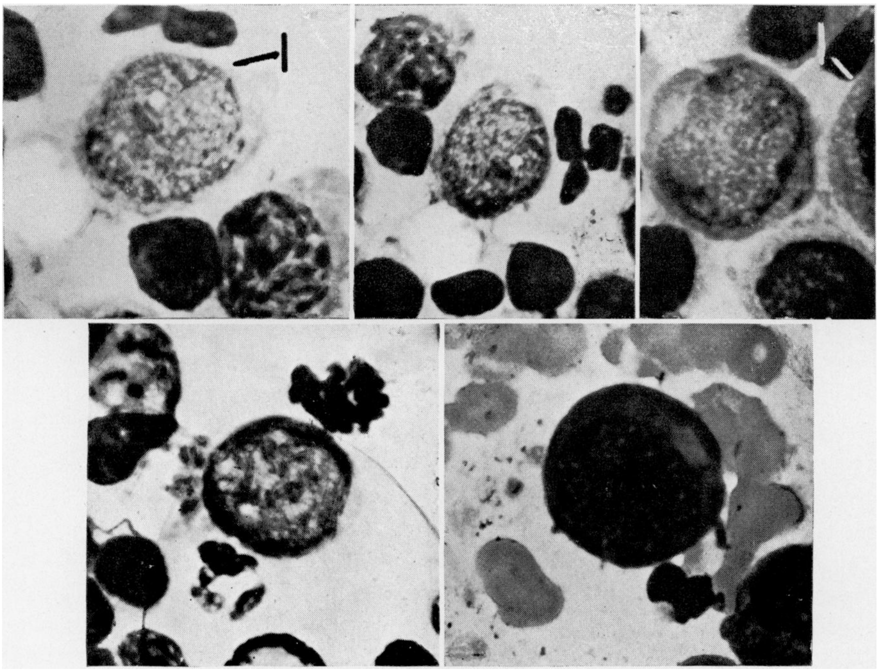

FIG. 4. Bone marrow from pig A showing immature erythrocytes which demonstrate the characteristics of the megaloblast.

would seem that a dietary factor probably played an important role in the development of the macrocytic anemia and associated bone marrow findings. It would be difficult to ascribe the hematologic findings to the direct effects of radiation. It may be that a combination of the two, plus possibly other undetermined factors, synergistically led to the development of such an anemia.

In any event, the finding of megaloblastic-like cells in the bone marrow of an animal manifesting a pronounced macrocytosis and a moderate anemia and achlorhydria is significant, and it is agreed with other investigators that swine may be a better animal in which to produce experimentally a pernicious anemia-like syndrome under controlled conditions.

Summary and conclusions

1. Hematological observations have been made on two swine for approximately seventeen months after exposure to atom bomb ionizing radiation at Bikini.

2. Macrocytic anemia and achlorhydria were found in both animals and a hyperplastic, "megaloblastic-like" bone marrow in one.

3. The experimental background of these swine with special reference to nutrition, radiation, age, and disease has been considered. However, it was not possible to evaluate conclusively the relative role that these conditions played in the production of the anemia and associated bone marrow findings.

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