# Anemia of Adjuvant-Induced Inflammation in Rats.* (32441)

J. N. Lukens, G. E. Cartwright, and M. M. Wintrobe
*Department of Medicine, University of Utah College of Medicine, Salt Lake City*

In a recent review of the anemia associated with chronic infections, rheumatoid arthritis and cancer in man(1), it was noted that the anemia is mild in degree and non-progressive in severitv. To understand the complex pathogenesis of this anemia, it is necessary to study the alterations in iron metabolism, red cell production, and red cell survival time not only during the early non-steadv state period when anemia is developing but also during the later period of steady state kinetics at the new equilibrium level. Unfortunately, a satisfactory experimental counterpart of the anemia of chronic disease in man has not been available. Although the alterations in iron metabolism which occur in this anemia have been produced in experimental animals with sterile turpentine inflammation(2,3) and by the injection of bacterial endotoxin(1,2,4) the changes are of short duration, anemia does not usually develop, and steady state kinetics do not apply. Chronic bacterial infections have been difficult to induce and maintain.

The purpose of this study is to evaluate "adjuvant-induced arthritis" in the rat as an experimental model for the anemia of chronic disorders in man. In this condition, a chronic inflammatory disease is induced in rats with a single intradermal injection of Freund's-type adjuvants(5,6,7). Following a latent period of 10 to 14 days, a generalized non-infectious inflammatory process is observed, characterized by migratory polyarthritis, generalized lymphoid hyperplasia, subcutaneous nodules, skin rashes, conjunctivitis and urethritis. The inflammatory reaction subsides gradually over a period of 60 to 70 days, leaving the animals with variable degrees of residual joint deformities.

*Methods.* Male Holtzman rats, weighing 350 to 450 g, were used in groups of five. The data are presented as the mean ± one standard error of such groups. Animals were caged individually and fed Purina laboratory chow *ad libitum*. Adjuvant, containing 0.6 mg dried heat-killed *Mycobacterium butyricum* finely dispersed in 0.1 ml of heavy mineral oil, was injected on day 0 into the right hind foot pad of 3 groups of rats. Four groups of rats served as controls. The control groups were sacrificed on days 0, 14, 32 and 60. The experimental groups were sacrificed on days 14, 32 and 60. The liver, spleen and left femur were excised *in toto* after the animals had been exsanguinated *via* the inferior vena cava under ether anesthesia.

Standard methods(8) were used for serial hematologic measurements on tail vein blood. Total red cell volume was measured with $Cr^{51}$ labeled rat red cells just before sacrifice(9). Plasma iron(10), total iron-binding capacity (11), and tissue iron(12) were measured by methods published previously. The iron content of ashed tissue was corrected for heme iron, quantitated as cyanmethemoglobin(13), in order to obtain a measure of tissue iron stores. Thin smears were prepared from femoral bone marrow dispersed in rat serum and stained to demonstrate iron according to the method of Rath and Finch(14). Sideroblast counts were performed on 200 normoblasts.

*Results.* A significant decrease in reticulocytes and in the volume of packed red cells (V.P.R.C.) was observed within 3 days following the administration of adjuvant (Fig. 1). Reticulocytes reached a minimal value on day 6, and thereafter, increased. Reticulocytosis of mild degree was a feature of adjuvant-induced inflammation between days 25 and 60. The V.P.R.C. decreased progressively over the first 17 days and then increased slowly between days 17 and 38. The V.P.R.C. remained relatively constant thereafter at about 48 ml/100 ml as compared with a value of 53 ml/100 ml in control animals (Fig. 1). The total red cell volume decreased progressively over the first 32 days and by

* This investigation was supported by a research grant (AM-04489) and a graduate training grant (2A-5098) from Nat. Inst. of Arthritis & Metab. Dis., N.I.H.

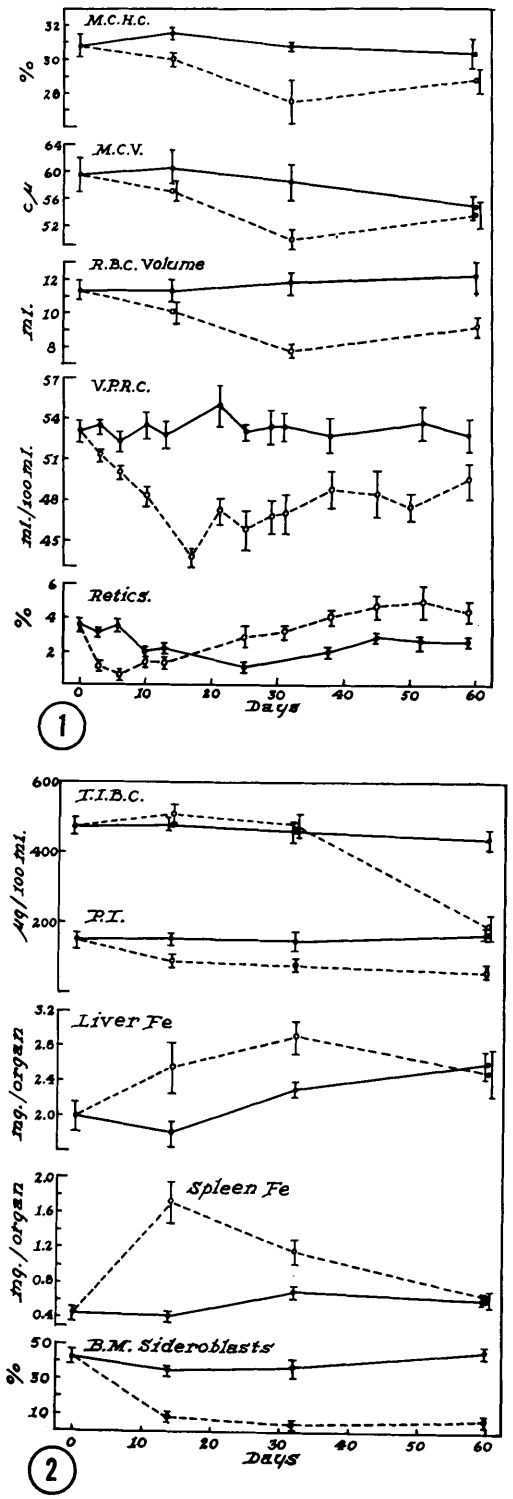


FIG. 1. Hematologic observations on control ( ● ) and adjuvant-injected ( ○ ) rats. The points and bars refer to means ± one standard error of means for groups of 5 rats. M.C.H.C., mean corpuscular hemoglobin concentration; M.C.V., mean corpuscular volume; R.B.C. volume, total red cell volume; V.P.R.C., volume of packed red cells; retics., reticulocytes.

FIG. 2. Distribution of iron in control ( ● ) and adjuvant-injected ( ○ ) rats (mean ± one S.E. for groups of 5 rats). T.I.B.C., total plasma iron binding capacity; P.I., plasma iron; Liver Fe, non-hemoglobin iron content of liver; Spleen Fe, non-hemoglobin iron content of spleen; B.M. sideroblasts, bone marrow sideroblasts (% of normoblasts containing siderotic granules).

the 60th day the value was still significantly less than in the control rats.

A few microcytic, hypochromic cells were seen in the smears of blood on day 14 but the proportion of such cells was not sufficient to significantly reduce the mean corpuscular volume (M.C.V.) and the mean corpuscular hemoglobin concentration (M.C.H.C.) until day 32 (Fig. 1). By the 60th day the M.C.V. had returned to within normal limits although the M.C.H.C. remained significantly decreased.

A decreased plasma iron concentration (P.I.) was observed when first measured on day 14 and remained below the control values throughout the entire experimental period (Fig. 2). The total iron-binding capacity (T.I.B.C.) remained within normal limits for the first 32 days but was markedly decreased on day 60. The saturation of transferrin with iron decreased from 34% to 19% on day 32. On day 60, because of the decrease in T.I.B.C., the % saturation was within normal limits.

The amount of non-hemoglobin iron in the spleen increased over the first 14 days and then decreased to within normal limits (Fig. 2). Liver non-hemoglobin iron increased progressively over the first 32 days and then decreased to within normal limits by day 60. The accumulation of iron was greater in the spleen than in the liver.

The proportion of normoblasts containing siderotic granules visible by light microscopy decreased from a value of 42% to 4%. Deficiency of normoblast iron contrasted sharply with the abundance of iron in marrow reticulum cells. Morphologically, reticulum cell iron of injected rats took the form of large, confluent masses (Fig. 3B) which replaced

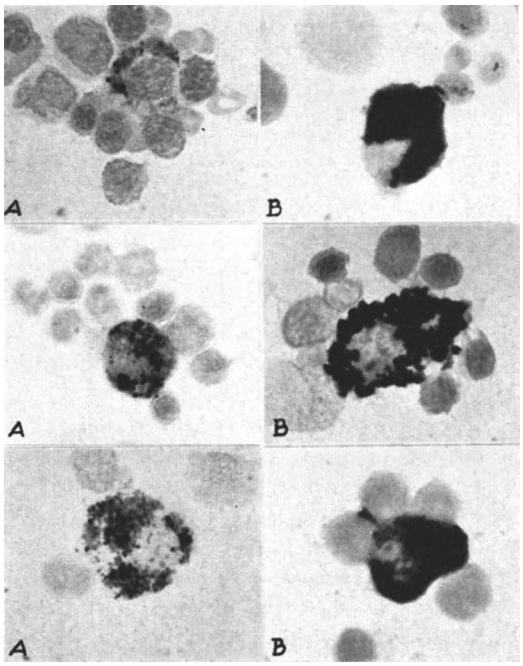


FIG. 3. Reticulum cell iron in bone marrow preparations from control (A) and adjuvant-injected rats (B).

the finely dispersed granules of iron observed in the cytoplasm of reticuloendothelial cells in preparations from control rats (Fig. 3A). The poverty of normoblasts for iron and the engorgement of reticulum cells with iron conglomerates constituted a distinctive pattern of iron distribution which was observed in marrow preparations from all adjuvant-injected rats.

*Discussion.* The mild, non-progressive anemia which developed in the rats following the intradermal injection of adjuvant resembled the anemia associated with chronic diseases in man in all important aspects(1). Morphologically, the anemia in man is usually normocytic, normochromic but may be hypochronic and occasionally microcytic. In the experimental anemia, the red cells were slightly microcytic and hypochromic. More importantly, the unusual pattern of iron metabolism consisting of hypoferremia, a decrease in transferrin, reduced saturation of transferrin with iron, and decreased bone marrow sideroblasts in the presence of normal or increased marrow reticuloendothelial iron which characterizes the anemia in man was also observed in the experimental anemia. This somewhat paradoxical situation, a relative marrow and red cell deficiency of iron in the presence of normal or increased reticuloendothelial iron in marrow, spleen and liver, is distinctive for this type of anemia and has been referred to as the "RE iron block."

An interesting aspect of the experimental anemia was the initial development of reticulocytopenia followed by a rapid decline in the V.P.R.C. It has not been possible to make similar observations in man. Following this period in the rats, the reticulocytes increased above control values and the anemia was partially but not completely alleviated. This suggests that marrow production was severely curtailed initially and then increased to provide an equilibrium state between production and destruction in which the rate of destruction was greater than normal and the rate of production was increased but not to a degree sufficient to entirely restore the V.P.R.C. to normal.

In man, during the phase when the anemia is constant in degree, the erythrocyte survival time is shortened and anemia develops because the bone marrow fails to increase its production sufficiently to compensate for the anemia(1,15). Although the erythrocyte survival time was not measured in the animals in this study, a similar mechanism is suggested by the persistent slight reticulocytosis in the presence of anemia from the 25th to 60th days. A reticulocytosis of similar degree has been observed in association with this anemia in man(15,16,17). Thus, it would seem that the two anemias may also be similar kinetically.

With the experimental model for the anemia of chronic disorders described above, it should be possible to investigate the pathogenesis of this anemia. Such a study should permit further characterization of the mechanisms which regulate the destruction of erythrocytes, control the production of erythrocytes, and regulate the supply of iron to the erythroid marrow.

*Summary.* The chronic, disseminated inflammatory reaction observed in rats following a single intradermal injection of Freund's-type adjuvant was associated with

a mild, non-progressive anemia which morphologically, biochemically and kinetically resembled the anemia associated with chronic disorders in man. It is suggested that "adjuvant disease" in the rat is an appropriate experimental model in which to study the complex pathogenesis of this distinctive but not uncommon form of anemia in man.

We wish to express our appreciation to Dr. R. Sidney Cloud for advice and assistance in the production of adjuvant disease and to Mrs. Alice Tustison and Mrs. Barbara Halliday for technical assistance.

1. Cartwright, G. E., Seminars Hemat., 1966, v3, 351.

2. Cartwright, G. E., Wintrobe, M. M., in Advances in Int. Med., Dock, W., & Snapper, I., ed., Year Book Publishers, 1952, v5, 165.

3. Quastel, M. R., Ross, J. F., Blood, 1966, v28, 738.

4. Kampschmidt, R. F., Upchurch, H. F., Johnson, H. L., Am. J. Physiol., 1965, v208, 68.

5. Pearson, C. M., Waksman, B. H., Sharp, J. T., J. Exp. Med., 1961, v113, 485.

6. Ward, J. R., Jones, R. S., Arthritis Rheum., 1962, v5, 557.

7. Waksman, B. H., Pearson, C. M., Sharp, J. T., J. Immunol., 1960, v85, 403.

8. Cartwright, G. E., Diagnostic Laboratory Hematology, 3rd Ed., Grune & Stratton, New York, 1963.

9. Reissmann, K. R., Nomura, T., Gunn, R. W., Brosius, F., Blood, 1960, v16, 1411.

10. Hamilton, L. D., Gubler, C. J., Cartwright, G. E., Wintrobe, M. M., Proc. Soc. Exp. Biol. & Med., 1950, v75, 65.

11. Ramsay, W. N. M., Clin. Chim. Acta, 1957, v2, 221.

12. Gubler, C. J., Brown, H., Markowitz, H., Cartwright, G. E., Wintrobe, M. M., Blood, 1952, v7, 1075.

13. Andersen, D., Shoemaker, W. C., Clin. Chem., 1965, v11, 442.

14. Rath, C. E., Finch, C. A., J. Lab. Clin. Med., 1948, v33, 81.

15. Bush, J. A., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M., J. Clin. Invest., 1956, v35, 89.

16. Nilsson, F., Acta Med. Scand., 1948, Supp'l. 210.

17. Strandberg, O., ibid., 1966, Suppl. 454.

Received July 5, 1967. P.S.E.B.M., 1967, v126.

## Isotope Abundances for Potassium of Biological Origin. (32442)

S. H. CHORLTON*, J. H. GREEN,† D. P. MELLOR‡ (Introduced by J. H. Heller)
*Department of Nuclear and Radiation Chemistry, School of Chemistry, Univ. of N.S.W., Sydney*

The isotopic composition of potassium from a wide variety of sources is now generally accepted as constant. A rare example of a departure from the normal isotopic composition is provided by the unusual concentration of potassium-40 in iron meteorites (1,2). Here the increase in concentration of this isotope is attributed to the action of cosmic radiation.

The earlier reports of fractionation of the isotope of potassium by biological system (3-9) must be accepted with reserve. Much of this work was done on samples that had not been purified. Later work on carefully purified samples yielded "constant" ratios(10-16) although the values of the ratios varied from author to author by 1-2%. The most recent measurements by Reutersward(12) (who had reviewed the earlier work) and Kendall(15) show that for samples which have not been purified, the isotope ratios are influenced by the impurities present.

It was mainly because of the earlier work of Lasnitski and Brewer(4,7,8) who found an increase in the $K^{39}$:$K^{41}$ isotope ratio for cancer tissues of human and animal origin and the more recent findings of Kendall(15) that impure samples of potassium from cancer tissue have a different isotope ratio from impure samples of potassium from normal tissue, that the present investigation was undertaken.

* Special Unit for Investigation and Research, Prince of Wales Hospital, Randwick, N.S.W. Australia.

† New England Inst. for Med. Research, Ridgefield, Conn.

‡ School of Chemistry, Univ. of N.S.W., Kensington, N.S.W., Australia.