EDITORIAL

"Trace" Metals in Blood, with Particular Reference to Zinc and Carbonic Anhydrase

The physiologic role of the trace metals was neglected in biochemical investigations of the blood in the last decade, but recently interest has been aroused in their function in blood formation. The term "trace metals" is unfortunate in that it has carried the implications that these elements cannot be measured quantitatively, that they probably occur accidentally, and that their presence is of no discernible consequence.

The development of precise microchemical methods has given the whole study new impetus even though many of these methods are complex and difficult. The needs of industry have given rise to the formulation of excellent colorimetric, flame photometric, polarographic, and emission spectroscopic technics, which are accurate for the measurement of very small quantities of elements, provided the necessary precautions regarding scrupulous cleanliness and avoidance of contamination of reagents and glassware are observed. Although the availability of radioactive isotopes has further stimulated interest in the field, it is erroneous to conclude that these substances serve their greatest usefulness in replacing microchemical technics; actually, the two serve best as mutually interdependent but supplementary approaches.

The investigation of iron metabolism has been for years an important part of hematologic research. The association of iron with hemoglobin and porphyrin pigments lends itself readily to spectrophotometric analysis, thus making it a rewarding subject for study, especially during periods when good microchemical technics for iron itself were not available. Knowledge concerning its metabolism is therefore developed to a degree unique among the metals. On the other hand, the relation of iron to the function of cytochromes, catalase and peroxidases in the erythrocyte is not established although it is known that these enzymes contain iron. Earlier physiologic work on cobalt and polycythemia has been re-examined recently in the light of new data on anemias, particularly in relation to vitamin B₁₂, a cobalt-containing complex. After previous work, copper, too, is being studied in relation to regeneration of hemoglobin and in regard to its role in the hemocuprein found in red blood cells. These elements are the only ones to have been studied with any degree of thoroughness. That titanium or vanadium are possible constituents of erythrocytes arouses no more than mild curiosity, although there is no reason for such an attitude other than the lack of information.

Available facts favor the assumption that many of the trace elements serve their function in association with protein molecules bound by S-H, COOH, NH₂ or porphyrin groups. These proteins may be involved in hormone or enzyme systems (including "vitamins") or may serve in functions of storage or transport. These metallo-enzyme systems are now receiving an increasingly large amount of attention.
An example of the rapid development of knowledge regarding a "trace" element is afforded by zinc; recent studies in the distribution and physiologic role of zinc are the result of advances in the chemistry of the rarer biologic elements and in nuclear physics and enzyme chemistry. It has been found that Zn is a constituent of human blood and is found in corpuscles and plasma. These studies were stimulated by the finding that Zn is more concentrated in leukocytes than in erythrocytes, as revealed by emission spectrography. Subsequently, measurements have been made by means of a colorimetric technic which is accurate to one microgram. The normal mean whole blood Zn level for males and females is 880 micrograms per cent, the normal mean plasma Zn 300 micrograms per cent, and 100 cc. of packed erythrocytes contain 1440 micrograms. Overall, 75 per cent of the whole blood zinc is found in the red cells, 22 per cent in the plasma and 3 per cent in the leukocytes separated from whole blood by a method based on physical chemical principles. While a greater fraction of the total blood Zn is contained in the erythrocytes, cell for cell the leukocytes contain 25 times as much as the erythrocytes. Statistical analysis of the data suggest that zinc is a physiologic constituent of blood in that its individual variations in concentration follow the pattern of commonly observed biologic distribution phenomena. Actually, the metal occurs in quantities in the body which in modern biologic language can hardly be called "traces."

Injection of radioactive $^{65}$Zn demonstrated its incorporation into the red and white blood cells of dog and man where it could be found as much as eight months after injection. Its passage across the placenta of the dog into the young has also been shown, and might indicate its physiologic need.

The nature of the protein to which Zn is bound in plasma is unknown at present, although preliminary investigations have shown that the metal becomes attached to the iron binding globulin in vitro. However, it is not known whether this is the transport mechanism in the body.

The role of zinc in leukocytes is a mystery at present. Its differential distribution among the various groups of white cells has not been studied. Attempts at radioactive tagging in order to study the leukocytic life span have at best been inconclusive. The possible occurrence of exchange of zinc across the white cell membrane contributes to the difficulties of interpretation, and, most important of all, the nature of the compound with which Zn is associated in the leukocyte is unknown. The decrease of Zn in the peripheral leukocytes of patients with chronic myelocytic, lymphocytic and monocytic leukemia is, however, a startling abnormality. The concentration of zinc in the leukocytes of these patients is approximately 10 per cent of that found in normal leukocytes. Under therapy with x-ray or urethane and in clinical remission, the falling leukocyte count is accompanied by a rise of zinc to normal levels. Attempts at raising the Zn level of these cells and lowering the leukocyte count by injections of stable zinc gluconate have not been successful. Whether leukemic cells are Zn deficient because they are immature, or whether they are leukemic because they are Zn deficient is difficult to evaluate at present. However, investigations of mouse epidermis have shown a decrease in
Zn and other elements as neoplasia developed. This is at least a clue to the fact
that there is a rearrangement of the "rarer elements" in neoplastic tissues. It is
evident that it will be necessary to study all of the minor elements in leukemic
cells before any conclusions concerning the true meaning of the decreased Zn con-
tent may be drawn. Unquestionably, however, studies of Zn metabolism offer a
new approach to leukopoiesis.

More is known concerning zinc in erythrocytes. Keilin and Mann\(^4\) recorded the
presence of that element in the enzyme carbonic anhydrase, shown earlier by
Meldrum and Roughton\(^1\) and by Stadie and O’Brien\(^2\) to be contained in the red
blood cell. The enzyme has a molecular weight of approximately 30,000 and con-
tains 0.3 per cent zinc. The functional significance of the zinc in the molecule is
shown by the fact that removal or inactivation of the metal by trichloroacetic acid
or by BAL\(^7\) also inactivates the enzyme. The enzyme catalyzes the reaction
\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \]
which otherwise would proceed at too slow a rate to permit life in mammals or birds. It is evident that carbonic anhydrase may be as important
in carbon dioxide transport as hemoglobin is in oxygen transport, yet the former
has not been investigated thoroughly. Studies of the enzyme in human and dog
blood have shown that all the activity is in the erythrocytes. There is none in
plasma or leukocytes. \textit{It is evident, therefore that zinc exists in blood in several states.}

The carbonic anhydrase content and Zn concentration of the red cells parallel
each other and vary directly with the hematocrit level and the hemoglobin con-
tcentration in congestive failure, anemia and polycythemia.\(^18\,\,22\) It is of unusual
interest, however, that in pernicious anemia the erythrocyte zinc concentration
and blood carbonic anhydrase activity are in or close to the normal range despite
low hematocrit cell percentages and hemoglobin values; the mean corpuscular
zinc concentration and carbonic anhydrase activity are increased several times
more than the high mean corpuscular hemoglobin and out of all proportion to
the increase in cell size. Normal findings develop in remission in a period of time
commensurate with the known mean life span of pernicious anemia red cells, evi-
dently as a result of their being replaced by normal cells.\(^19\,\,22\) In other clinical
conditions such as the postnatal state in infants,\(^23\) the sickling phenomenon\(^24\)
and paroxysmal cold hemoglobinuria\(^25\) studies of the carbonic anhydrase system
suggest a role of the enzyme in their mechanisms.

Although the above findings relating to disturbances in enzyme activity and
zinc concentration in various conditions are of interest, their significance cannot
be stated. At present, sufficient data are not available on the physiology of car-
bonic anhydrase to make possible a definition of its precise function in the blood.
Thus, although it is known what the enzyme can do, what it actually does is not
known. However, the observation that a close parallelism exists between the
erthrocyte carbonic anhydrase activity and zinc content in all conditions studied\(^19\)
provides a useful method for estimating the \textit{amount} of the enzyme in the red blood
cells; apparently all the zinc in erythrocytes is part of the carbonic anhydrase
molecule.

The present data bearing on zinc in the blood, although incomplete, call atten-
tion to the even larger gaps which exist in knowledge of other erythrocyte metals and metallo-enzyme systems.

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REFERENCES

* Senior Research Fellow, National Research Council, Committee on Growth, supported by the American Cancer Society.
EDITORIAL


NEWS AND VIEWS

BLOOD CLUB

The second annual meeting of the Blood Club will be held in Atlantic City on May 1, 1949 on the Sunday evening just prior to the meetings of the American Society of Clinical Investigation and the Association of American Physicians. Dinner will be served promptly at 6:30 P.M. in Haddon Hall Hotel after which the following preliminary program, in the form of a panel discussion on Hemorrhagic Disorders Associated with Defective Coagulation, has been arranged:

A. Introduction.
B. Discussion of the Abnormal Mechanisms in Coagulation in the Following Conditions:
   1. Hypoprothrombinemia.
      a. Idiopathic and/or Congenital
      b. Secondary to Intestinal Absorption and/or Dietary Deficiency
   2. Deficiencies in Platelet Material.
      a. Thrombocytopenia
      b. Thrombasthenia
   3. Hemophilia.
      Discussors: K. M. Brinkhous, C. G. Craddock, F. L. Munro, L. M. Tocantins
   4. Deficiency in Accelerator Globulin
      Discussors: K. M. Brinkhous, W. H. Seegers
   5. Circulating Anti-Coagulants.
      a. Hemophilia
      b. Idiopathic
      c. Radiation
      d. Nitrogen Mustard
      e. Anaphylactic Shock
      f. Secondary to Heparin
      g. Secondary to Dicumarol
      Discussors: J. Garrott Allen, C. L. Conley, C. G. Craddock, Leon Jacobson, F. L. Munro

This meeting is open to all interested physicians. Reservations for the dinner must be made by writing to Dr. Lawrence. The dinner charge will be at regular hotel prices.

The Committee: John S. Lawrence, University of California, at Los Angeles
Frank Bethell, Ann Arbor
Joseph F. Ross, Boston.
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