Technical Papers

An Iron-binding Component in Human Blood Plasma

ARTHUR L. SCHADE and LEONA CAROLINE
Overly Biochemical Research Foundation, Inc.
New York City

Following the demonstration of a protein component in raw egg white capable of binding iron (*5*), we investigated fractions of human plasma for a similar property.

Through the courtesy and interest of E. J. Cohn, L. E. Strong, and their associates we obtained representative samples of plasma fractions prepared by them (*2*).[1] Fraction I (fibrinogen), Fraction II-1 (γ-globulin), Fraction II + III (prothrombin, immune globulins, isohemagglutinins, Rh antibodies, carotenoids, and phosphatides associated with globulins), Fraction IV-1 (lipids apparently associated with β-globulins), Fraction IV-3,4 (rich in α- and β-globulins), and Fraction V (albumin), after addition to nutrient broth in a concentration approximately equal to that found in whole plasma, were tested for their ability to inhibit growth of *Shigella dysenteriae*. Of these fractions, only II + III and IV-3,4 were clearly active. The tubes containing Fraction V showed slightly delayed growth. When excess ferrous iron was added to the inhibited cultures, only those tubes with Fraction IV-3,4 showed bacterial development after an additional 18-hour incubation. Qualitative tests for the formation of a typical salmon pink color (*5*) upon addition of ferrous iron to solutions of Fractions II + III and IV-3,4 indicated that only Fraction IV-3,4 was active in this respect. Hence, qualitatively, Fraction IV-3,4, as shown by the biological and colorimetric tests, is similar to the active egg white component as regards affinity for and reaction with iron.

When ferrous iron as ferrous ammonium sulfate was added to Fraction IV-3,4 in 0.02 M phosphate buffer at pH 6.5, it was found by colorimetry that 1 mg. of protein [N × 7.13 (*4*)] took up a maximum of 0.44 γ Fe^{++}. In the biological test in which graded amounts of iron were added to inoculated nutrient broth cultures of *S. dysenteriae* to observe at what iron level growth took place, the results showed that 1 mg. of protein made 0.38 γ Fe^{++} unavailable for bacterial development. Other samples of Fraction IV-3,4 and a subfraction, IV-3, gave similar results, although the ratio of milligrams of protein to gammas of iron bound varied from the example given.

Since our work with partially fractionated egg white gave us material whose activity approached that of 1 mg. of protein for 1 γ Fe^{++}, we sought to raise the activity of Fraction IV-3,4 by further fractionation. For this purpose samples of the fraction were dialyzed against several changes of distilled water until a precipitate had formed which, on analysis, represented approximately one-third of the nondialyzable nitrogen. To test the precipitate for its capacity to form a colored product with added iron, it was first suspended in 0.25 per cent saline solution and finally dissolved through alkalinization with sodium carbonate. The resultant opalescent solution was then neutralized with hydrochloric acid and mixed with graded amounts of iron. No color whatever developed, even when the ratio was as great as 1 mg. of protein to 1.5 γ of iron. Contrariwise, when the nonprecipitated fraction was tested colorimetrically with added iron, it was found that saturation of the active protein component occurred when 0.66 γ Fe^{++} was added to 1.0 mg. of protein of the sample. Fig. 1 illustrates this result.

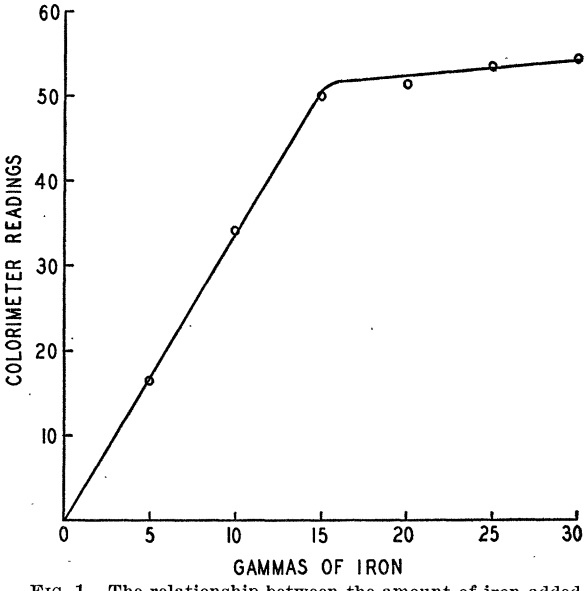

FIG. 1. The relationship between the amount of iron added to human plasma Fraction IV-3,4 and color production. Five cc. of the soluble portion of the dialyzed Fraction IV-3,4, adjusted to pH 6.4 with $NaHCO_3$ and containing 25.6 mg. of protein, were set at zero in a Klett-Summerson colorimeter with the blue filter #42. Additions of 5 γ Fe^{++} in 0.1-cc. amounts were made to the sample. Four minutes after each addition, readings were made of the increase in depth of the characteristic salmon pink color.

[1] The products of plasma fractionation employed in this work were developed by the Department of Physical Chemistry, Harvard Medical School, under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Harvard University.

When the soluble dialyzed fraction was tested biologically, the results showed that approximately 0.57 γ of iron were made unavailable for the growth of *S. dysenteriae* by 1 mg. of the protein in nutrient broth. Since the soluble nitrogen represented approximately two-thirds of the total nitrogen of the dialyzed sample, it should be noted that the activity of this fraction in terms of iron-binding power was proportionately greater and accounted for all of the activity shown by the undialyzed fraction, IV-3,4.

The presence in human plasma of a protein fraction having the capacity to bind iron at physiological pH's may have some relevance to the problem of the regulation of iron absorption from the intestinal mucosa as well as that of iron transport by the blood through the body (*3*).[2] In this connection it is of some interest to note that for a medium-sized man of 70-kg. weight it is calculated that, through the intervention alone of the amount of active fraction in plasma when saturated with iron, as much as 9 mg. of iron (0.26 mg./100 cc. plasma) could be carried by the blood stream at any given moment. Analyses of iron content of normal plasma have given values of 0.1–0.3 mg./100 cc. under ordinary conditions (*1*). Of no less importance is the possible significance of this iron-binding fraction in the blood stream for the bacteriostatic action it exerts upon iron-sensitive pathogens as shown by the *in vitro* studies with *S. dysenteriae*. What value an iron-saturated solution of this plasma fraction may have for iron administration in certain anemias remains to be demonstrated.

References

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Origin of Nitrogen in Natural Gases

George H. Cady

Department of Chemistry and Chemical Engineering
University of Washington, Seattle

About three years ago W. B. Lang (*3*) discussed the occurrence of nitrogen in natural gases and suggested that the probable source of the element could be established by analyzing suitable samples for nitrogen and the rare gases, argon, krypton, xenon, and neon. If the relative proportions of nitrogen and argon in a particular gas sample were found to be substantially the same as in air, one might reasonably conclude that the nitrogen and argon had originally been present in the atmosphere and had in some way become trapped within the earth. On the other hand, an abundance ratio of nitrogen to argon much higher than that for air might mean that the nitrogen in the gas had been produced within the earth by chemical reactions.

Similar deductions from gas analyses have been made in the past by others, and significant contributions to the literature about this topic have been made (*1, 4–9*). The interpretation of the data has not always been exactly the same as that of Lang. For example, Moureu was of the opinion that the substantially fixed relative proportions of nitrogen, argon, krypton, and xenon which he and A. Lepape (*5*) found in the atmosphere and in gases from within the earth could be explained by the hypothesis that these inert elements had not been separated from each other by natural processes and were therefore present in all terrestrial gases in the proportions established at the time of creation.

TABLE 1

Proportions by Volume of Nitrogen and Helium Group Gases in Certain Natural Gases

Sample No.	% by volume of N_2	% by volume of He	$\frac{N_2/A \text{ in gas}}{N_2/A \text{ in air}}$	$\frac{(Kr+Xe)/A \text{ in gas}}{(Kr+Xe)/A \text{ in air}}$
I	72.9	7.55	0.98	0.4
II	52.6	6.00	1.19	0.8
III	24.7	1.73	1.50	3.0
IV	96.8	0.047	4.0	2.9
V	30.6	0.19	3.0	3.0
VI	10.0	0.052	3.3	3.7
VII	28.0	0.55	2.4	3.0
IX	97.0	0.074	1.25	.
X	24.1	0.305	8.2	0.15

A few analyses of the type proposed by Lang (*3*) were made as a part of a study recently published by the author and his father, H. P. Cady (*2*). In this work the helium group gases and nitrogen were determined in a few natural gases of high nitrogen content. Data for the different samples are presented in Table 1. The sample numbers used are the same as those employed in the earlier publication (*2*), where one may find detailed descriptions of the gases.

The wide variation in the (krypton + xenon)/argon ratios shown in the table suggests that the rare gases have become partially separated from each other by natural processes. It is therefore possible that argon and nitrogen have also become separated from each other and consequently occur in different proportions in different gases. Since this is the case and since there is no assurance that the inert elements in natural gas originally came from the atmosphere rather than from within the earth, one cannot use the available

[2] Since the time this manuscript was submitted, an article by Holmberg and Laurell (*Acta Physiol. Scand.* (Sweden), 1945, **10**, 307–319) concerning the regulation mechanism of serum iron has become available. This article refers to a thesis by Vahlquist (*Das Serumeisen* (Diss.). Uppsala: 1941), whose data suggested that serum iron is bound to both the albumin and, especially, the globulin protein fractions.