When hemoglobin H was added to serum, only a free band of hemoglobin was observed (Fig. 1). This band sometimes split into two components of nearly the same mobility, which were probably the isomeric forms of hemoglobin H (*14*).

Formation of the hemoglobin-haptoglobin complex was not demonstrated when hemoglobin Bart's was used. Some barely discernible benzidine-positive bands observed in the position of the haptoglobin band in a few runs probably represented impurities in the preparations of hemoglobin Bart's. The manipulation of hemoglobins H and Bart's did not appear to account for the absence of binding since hemoglobin A, which had been prepared from the same electrophoretic separations, exhibited an apparently normal haptoglobin-binding capacity. Further evidence of the failure of hemoglobins H and Bart's to form complexes with haptoglobins was derived from the absence of retardation of migration of the haptoglobins on gels stained with amido black. (The haptoglobin-hemoglobin complex migrates faster toward the cathode than the corresponding haptoglobin bands of serum.)

The possibility that globin from the abnormal hemoglobins was blocking the haptoglobin was considered. However, when hemoglobin A was added to serums that had been previously incubated for 15 minutes at 20°C with hemoglobin H or Bart's, the normal hemoglobin-haptoglobin pattern was observed on electrophoresis.

The formation of the hemoglobin-haptoglobin complex was also studied by means of starch gel electrophoresis, with 0.003*M* phosphate buffer (*13*) at *p*H 7.0, where hemoglobin H failed to bind haptoglobin. At *p*H 7.0, hemoglobin Bart's migrated near the haptoglobin band and definite conclusions concerning formation of the complex could not be drawn.

The peroxidase activity of hemoglobins H and Bart's did not increase in the presence of serum, while in hemoglobin A prepared in the same manner a clear increase in peroxidase activity as a consequence of the formation of the haptoglobin-hemoglobin complex was demonstrated.

Neither the structural sites nor the groups involved in the formation of the haptoglobin-hemoglobin complex have been defined. In the studies reported here, two hemoglobins which were tetramers of a single type of polypeptide chain (β_4^A and γ_4^F) failed to bind haptoglobin, although normal hemoglobin A (α_2^A β_2^A), A_2 (α_2^A $\delta_2^{A_2}$), or F (α_2^A γ_2^F) containing β-, δ-, or γ-chains regularly bound at least the common types of human haptoglobins. The failure of β- or γ-tetramers to bind haptoglobin has two obvious alternative explanations: (i) that the presence of α-polypeptide chains is necessary for the binding, or (ii) that the altered configuration demonstrated for hemoglobin H (*5*) and presumed for hemoglobin Bart's results in loss of the haptoglobin binding capacity.

The available evidence does not permit us to decide conclusively which of these possibilities is correct. Studies by others have indicated that electrostatic and van der Waal's forces (*15*) [but probably not SH groups (*16*)] are important in the formation of the complex, but there is little evidence concerning the contribution of the individual hemoglobin polypeptide chains in the complex formation. Single changes in the amino acid sequence of either chain (for example, hemoglobins I^α or S^β) do not interfere with the formation of the haptoglobin-hemoglobin complex. The greater acidic charge of hemoglobin H or hemoglobin Bart's would not account for the failure to bind haptoglobin, since hemoglobin I, which has about the same charge as hemoglobin H, formed the hemoglobin-haptoglobin complex.

At this time there is no method for testing the possibility that α-chains as they occur in the normal hemoglobin-A tetramer are necessary for the formation of the hemoglobin-haptoglobin complex. The α^A-chains that have been isolated (*17*) are monomers and consequently have great changes in configuration. We have, however, been unable to demonstrate complex formation between such α^A-chains prepared in our laboratory and haptoglobin.

The formation of the hemoglobin-haptoglobin complex probably represents a binding between two complementary surfaces as visualized by Pauling (*18*). The failure of hemoglobins H or Bart's to bind haptoglobin is related either to configurational alterations or to the absence of the α^A-chain dimers in these hemoglobins.

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References and Notes

1. M. F. Jayle and J. Moretti, *Prog. Hematol.* **3**, 343 (1962).
2. A. A. van Royen, thesis, Univ. of Delft, Delft, Netherlands (1950).
3. A. G. Bearn and E. C. Franklin, *Science* **128**, 586 (1958); E. Alfonso and J. Proenca, *Nature* **198**, 1100 (1963).
4. C. Baglioni, *Proc. Natl. Acad. Sci. U.S.* **48**, 1880 (1962).
5. M. F. Perutz and L. Mazzarella, *Nature* **199**, 639 (1963).
6. D. L. Drabkin, *Arch. Biochem. Biophys.* **21**, 224 (1949).
7. J. Reed and H. M. Ranney, in preparation.
8. H. G. Kunkel, R. Ceppellini, V. Müller-Eberhard, J. Wolf, *J. Clin. Invest.* **36**, 1615 (1957).
9. D. W. Allen, W. A. Schroeder, J. Balog, *J. Am. Chem. Soc.* **80**, 1628 (1958).
10. M. Nyman, *Scand. J. Clin. Lab. Invest. Suppl.* **39**, 1 (1959).
11. M. D. Poulik, *Nature* **180**, 1477 (1957).
12. O. Smithies, *Biochem. J.* **71**, 585 (1957).
13. C. B. Laurell and C. Grönvall, *Advan. Clin. Chem.* **5**, 135 (1962).
14. R. Benesch, R. E. Benesch, H. M. Ranney, A. S. Jacobs, *Nature* **194**, 840 (1962).
15. L. Robert, V. Bajic, M. F. Jayle, *Compt. Rend.* **242**, 2868 (1956).
16. L. Robert, G. Boussier, M. F. Jayle, *Experientia* **13**, 111 (1957).
17. E. R. Huehns, E. M. Shooter, N. Dance, *Biochem. Biophys. Res. Commun.* **5**, 362 (1961).
18. L. Pauling, *Discussions Faraday Soc.* **13**, 170 (1953).
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Iron Absorption: The Effect of an Iron-Deficient Diet

Abstract. *A diet deficient in iron causes a rapid, marked increase in iron absorption in rats. The increased absorption occurs in the absence of a significant change in iron stores as judged by the effect of an equivalent change in stores produced by phlebotomy, and in the absence of increased erythropoietic activity as judged by the rate of removal of iron-59 from the plasma.*

Many experiments suggest that the intestinal mucosa's absorptive capacity for iron is controlled by erythropoietic activity and by iron stores (*1*). Experiments with rats have suggested to us that the iron in the normal diet is an additional controlling factor.

We have studied iron absorption in male albino rats, using radioactive iron (Fe^{59}) with 250 μg of carrier iron and estimating retention of the isotope by counting the residual radioactivity in the total body (*2*). The investigation reported here began with the chance observation of strikingly increased iron

Table 1. Absorption of Fe^{59} in rats maintained on iron-deficient and normal diets.

Diet	No. of days*	Absorbed (% ± S.D.)	No. of rats	P†
Iron-deficient	1	21.9 ± 5.5	10	
Normal		19.9 ± 8.6	10	>.1
Iron-deficient	2	24.0 ± 11.5	11	
Normal		18.0 ± 7.6	11	>.1
Iron-deficient	3	28.6 ± 8.6	10	
Normal		24.5 ± 6.1	10	>.1
Iron-deficient	4	38.7 ± 10.1	12	
Normal		18.7 ± 6.1	10	<.001
Iron-deficient	13	59.3 ± 23.4	11	
Normal		12.7 ± 4.4	11	<.001

* The number of days on which the rats were fed the diet. The number of days of iron deprivation is one more than the number indicated, as the animals were fasted for 24 hours prior to testing the absorption of iron with Fe^{59}. † Statistical comparison is made by using the two-tailed "*t*" test.

absorption in rats maintained on iron-deficient diets for short periods of time. The diet, except for the absence of iron, is nutritionally adequate and consists of casein, sucrose, added vitamins, fat, and minerals. The iron content is 3.9 μg per gram of diet, which is approximately 2 percent of the iron content of the rat diet normally used in our laboratory (*3*).

Systematic study of iron absorption in rats on an iron-deficient diet showed that there was no change in the amount of iron absorbed for the first 4 days of iron deprivation, that there was an increased iron absorption on the 5th day, and that the amount absorbed was markedly increased by 14 days after beginning the iron-deficient diet (Table 1).

The rat loses approximately 0.23 percent of its total body iron per day, and total body iron is approximately 4.2 mg per 100 g of body weight (*4*). At this rate the total amount of iron lost during a 7-day period of iron deprivation, assuming zero iron absorption, would be no greater than 0.15 mg in a 219-g rat. Blood containing this amount of hemoglobin iron was removed from each of a group of rats (mean weight 219 g) by bleeding from the orbital plexus, after which the rats were maintained on a standard diet containing iron. Iron absorption was tested 6 days after bleeding, but no increase in the amount absorbed was demonstrated. Nine control rats absorbed 16.3 ± 4.6 percent of the given dose. Eleven experimental rats absorbed 15.4 ± 5.7 percent of the given dose.

Table 2. The half-life ($T_{½}$) of Fe^{59} in the plasma of rats on iron-deficient diets.

Control		Experimental	
$T_{½}$ (min)	Plasma iron (μg/100 ml)	$T_{½}$ (min)	Plasma iron (μg/100 ml)
66		70	135
56		70	173
66		63	126
66		56	
75	143	69	
68	135	70	
66	168	89	248
72	139	89	176
		75	
		73	190
Mean*			
67		72	

* A *t*-test comparison of the two groups shows no significant difference between the two means ($p > .1$).

The rate of removal of Fe^{59} from the plasma was studied in another group of rats after 7 days of iron deprivation (including 1 day of fasting). The rate in the rats on the iron-deficient diet was similar to the rate in the rats on the normal diet (Table 2).

We considered the possibility that some attribute of the diet, other than the lack of iron, might have caused the increase of iron absorption. Iron was added to the experimental diet in an amount sufficient to equal the iron content of the standard laboratory rat diet (*3*). A comparison of iron absorption in rats maintained on this mixture for 1 week with rats maintained on a normal diet showed no difference. Twelve control rats absorbed 18.9 ± 7.9 percent of the given dose. Twelve experimental rats absorbed 17.8 ± 6.1 percent of the given dose.

An iron-deficient diet appears to increase iron absorption without appreciably changing the total amount of iron stored in the body, as judged by the effect of an equivalent change produced by phlebotomy, and without stimulating erythropoiesis, as judged by the half-life ($T_{½}$) of Fe^{59} injected into the plasma. The possibility exists that an iron-deficient diet may result in prompt depletion of iron in the intestinal mucosa, and that this may be responsible for the increase in iron absorption (*5*). However, the 5-day interval required for iron deprivation to have any effect in increasing iron absorption suggests that the mechanism is more complicated than a direct interaction between luminal iron and the absorptive epithelium because the total population of cells in the mucosal epithelium is replaced in 1.6 days (*6*).

It appears that a partial block to the absorption of a small amount of iron is effected by the iron content of the normal diet. The existence of the block is best appreciated by removing iron from the diet. Since large doses of iron do not appreciably further inhibit iron absorption (*7*), the degree of inhibition would appear to be already near the maximum with a normal diet. The iron content of the rat diet is high as judged by human dietary standards, so that the applicability of these observations to iron absorption in human subjects remains to be proven.

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References and Notes

1. T. H. Bothwell, G. Pirzio-Biroli, C. A. Finch, *J. Lab. Clin. Med.* **51**, 24 (1958); M. Field, M. Seki, M. L. Mitchell, T. C. Chalmers, *ibid.* **55**, 929 (1960).
2. S. Pollack, R. Kaufman, W. H. Crosby, J. E. Butkiewicz, *Nature* **199**, 384 (1963).
3. Rat and Mouse Diet, D & G Dog Foods and Laboratory Animal Foods, Frederick, Md. This diet contains 186 μg of iron per gram.
4. R. M. Kaufman and S. Pollack, in preparation.
5. M. E. Conrad, L. R. Weintraub, W. H. Crosby, *J. Clin. Invest.*, in press.
6. C. P. Leblond and C. E. Stevens, *Anat. Record* **100**, 357 (1948).
7. E. B. Brown, R. Dubach, C. V. Moore, *J. Lab. Clin. Med.* **52**, 335 (1958).

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Rat Electroretinogram: Evidence for Separate Processes Governing b-Wave Latency and Amplitude

Abstract. *Studies of the simultaneous changes in the latency and amplitude of the b-wave of the rat electroretinogram (ERG) under three different conditions of adaptation show that the latency is primarily a function of the absolute stimulus intensity, being only slightly affected by conditions that strongly reduce the amplitude. This implies that the latency and the amplitude are determined by two independent processes, with the latency-process more closely linked to the initial photochemical events. Furthermore, the different adaptation conditions have surprisingly similar effects on the shape and amplitude of the electroretinogram, which suggests that they all produce only one type of variation in the amplitude-determining process.*

It is well known that in the dark-adapted eye, the latency and amplitude of the b-wave of the electroretinogram (ERG) vary oppositely. When the stimulus-flash intensity is raised, the amplitude increases but the latency