the pressure. The chemical reactions involved in the formation of ferromanganese nodules are of the type:

$$Mn^{++} + \tfrac{1}{2}O_{2(g)} + 2OH^- = \delta MnO_2 + H_2O \quad (1)$$

$$5Mn^{++} + 2O_{2(g)} + 10\ OH^- = 4MnO_2 \cdot Mn(OH)_2 \cdot 2H_2O + 2H_2O \quad (2)$$

$$4MnO_2 \cdot Mn(OH)_2 \cdot 2H_2O + \tfrac{1}{2}O_2 = 5\ \delta MnO_2 + 3H_2O \quad (3)$$

The formula $4MnO_2 \cdot Mn(OH)_2 \cdot 2H_2O$ represents the 7-Å manganite phase as suggested by Feitknecht and Marti (*13*) and by Buser, Graf, and Feitknecht (*14*). The 10-Å manganite can be represented by $3MnO_2 \cdot Mn(OH)_2 \cdot 3H_2O$ (*8*).

It can be seen that the only species whose activity is subject to change with depth in Eq. 3 is oxygen. The equilibrium partial pressure of oxygen will increase with increasing hydrostatic pressure (*15*). Also, the oxygen content of seawater tends to remain constant or increase with depth between 1000 and 5000 m in the Pacific Ocean (*16*). As a result of these factors, the formation of δMnO_2 would be favored at greater depths. This is contrary to the data presented here.

The two other parameters which can cause ΔG to change as a function of depth are temperature and pressure. The change of ΔG with temperature is given by:

$$\left(\frac{\partial \Delta G}{\partial T}\right)_P = -\Delta S$$

where ΔS refers to the entropy change of the reaction. Figure 5 shows that the most probable occurrence of δMnO_2 is at 1000 to 1500 m, and that of the manganite phases is at 4000 to 4500 m. The temperature difference represented by this depth difference is usually 3° or 4°C. Therefore, the entropy change must be large for this free energy change to be of significance.

On the other hand, the pressure change is 300 atm. The effect of pressure on ΔG is:

$$\left(\frac{\partial \Delta G}{\partial P}\right)_T = \Delta \bar{v}$$

where $\Delta \bar{v}$ is the partial molal volume change during the reaction. This latter parameter seems to be the most promising for consideration of controlling the mineralogy of ferromanganese nodules. It has sufficient magnitude and universality to control the mineralogy on an ocean-wide basis. At present this hypothesis cannot be tested because of a lack of molal volume measurements of the reactants and products.

It should be noted that the measured rate of accumulation (*17*) roughly corresponds to rates which can be calculated from laboratory kinetic studies (*18*). However, at present there exist inadequate data to ascertain the effect of temperature and particularly pressure on reaction kinetics of nodular formation.

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References and Notes

1. E. D. Goldberg, *J. Geol.* **62**, 249 (1954).
2. J. P. Riley and P. Sinhaseni, *J. Marine Res. Sears Found. Marine Res.* **17**, 466 (1958).
3. R. G. Burns and D. W. Fuerstenau, *Am. Mineralogist* **51**, 895 (1966).
4. N. S. Skorniakova and P. F. Andurshchenko, *Lithol. Mineral Resources* **5**, 21 (1964).
5. H. W. Menard, *Marine Geology of the Pacific* (McGraw-Hill, New York, 1964), pp. 183–188.
6. J. L. Mero, *The Mineral Resources of the Sea* (Elsevier, Amsterdam, 1965), pp. 225–231.
7. G. Arrhenius, J. L. Mero, J. Korkish, *Science* **144**, 170 (1964).
8. W. Buser and A. Grütter, *Schweiz. Mineral. Petrog. Mitt.* **36**, 49 (1956).
9. E. D. Goldberg, in *Chemical Oceanography*, J. P. Riley and G. W. Skirrow, Eds. (Academic Press, London, 1965), vol. 1, pp. 163–196.
10. D. F. Hewitt, M. Fleischer, N. Conklin, *Econ. Geol.* **58**, 1–51 (1963); K. J. Murata and R. C. Erd, *J. Sediment. Petrol.* **34**, 633 (1964); F. T. Manheim, *Narragansett Marine Lab., Univ. Rhode Island, Occasional Publ. No. 3*, pp. 217–275 (1965).
11. L. G. Skillén, in *Oceanography*, M. Sears, Ed. (AAAS, Washington, D.C., 1961), pp. 549–582.
12. S. R. Taylor, *Geochim. Cosmochim. Acta* **19**, 100 (1960).
13. W. Feitknecht and W. Marti, *Helv. Chim. Acta* **28**, 148 (1945).
14. W. Buser, P. Graf, W. Feitknecht, *ibid.* **37**, 2322 (1954).
15. I. M. Klotz, *Limnol. Oceanog.* **8**, 149 (1963); T. Enns, P. F. Scholander, E. D. Bradstreet, *J. Phys. Chem.* **69**, 389 (1965).
16. J. L. Reid, Jr., *Intermediate Waters of the Pacific Ocean* (Johns Hopkins Press, Baltimore, 1965).
17. E. D. Goldberg, in *Oceanography*, M. Sears, Ed. (AAAS, Washington, D.C., 1961), pp. 583–597; M. L. Bender, W. S. Broecker, T. Ku, *Science* **151**, 325 (1966); S. Barnes and J. Dymond, *Nature* **213**, 1218 (1967).
18. J. J. Morgan and W. Stumm, *The Proceedings of the Second International Water Pollution Research Conference, Tokyo* (Pergamon Press, New York, 1964), pp. 103–131.
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Hemoglobin Portland 1: A New Human Hemoglobin Unique in Structure

Abstract. *A new hemoglobin (Hb), Portland 1, has been found in a newborn infant having multiple congenital anomalies and complex autosomal chromosomal mosaicism. The new hemoglobin has a unique tetrameric structure (molecular weight, 66,000) composed of two pairs of different types of chains, neither of which is* α, $\gamma_2 x_2$. *The x-chain of Hb Portland 1 may be a new type of hemoglobin chain, but the available evidence suggests that it may be identical with the* ϵ *chain. We suggest that Hb Portland 1 is an embryonic hemoglobin that persisted until after birth in relatively large amounts in this patient.*

A new hemoglobin (Hb), Portland 1, was discovered (*1*) in a female Chinese infant having multiple congenital anomalies; chromosomal studies of lymphocytes revealed complex mosaicism (normal/trisomy 16/short arm 17-18 deletion) (*2*). The blood sample used by us was obtained immediately after the infant's death at 20 days. The washed erythrocytes were hemolyzed with distilled water and toluene (*3*), and the clarified hemolyzate was subjected to starch-gel electrophoresis in tris–ethylenediamine tetraacetic acid–borate buffer, *p*H 8.15 (*4*). Comparison of the electrophoretic pattern of this hemolyzate with those of several other hemoglobin specimens (Fig. 1) shows that a hemoglobin component having an electrophoretic mobility greater than Hb-A but less than Hb Bart's is present in the hemolyzate from the propositus. The name Portland 1 is proposed for this previously undescribed hemoglobin, which represented approximately 5 percent of the total hemoglobin; the remainder included Hb Bart's (5 percent), Hb-F_I (11 percent) (*5*), Hb-F_{II} (55 percent), and Hb-A (22 percent). It is important to note that no Hb Portland 1 was demonstrable in the parents of the propositus.

Structural characterization of Hb Portland 1 was carried out by initial isolation of the hemoglobin components by use of IRC-50 ion-exchange chromatography (phosphate buffer, *p*H 6.7, 0.05*M* Na^+, 0.01*M* KCN) (*6*) followed by further purification employing starch-block electrophoresis (barbital buffer, *p*H 8.6, 0.5*M*) (*7*).

The $s_{20,w}$ (4.45*S*) and $D_{20,w}$ (6.54 ×

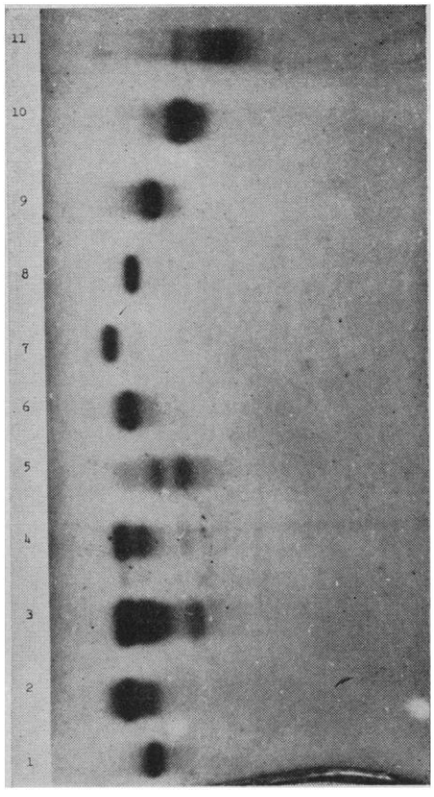

Fig. 1 (left). Starch-gel electrophoresis with tris–ethylenediamine tetraacetic acid–borate buffer, *p*H 8.15; the Hb components present in each specimen are read from left to right: (1) pure Hb-A; (2) Hb-F + Hb-A; (3) hemolyzate of propositus, Hb-F + Hb-A + Hb Portland 1 + Hb Bart's; (4) same as (3) but lower in concentration; (5) Hb Portland 1 + Hb Bart's; (6) Hb-F_I from IRC 50 chromatography; (7) Hb-F_{II} from IRC 50 chromatography; (8) pure Hb-A; (9) pure Hb Portland 1; (10) pure Hb Bart's; (11) Hb Bart's + Hb-H.

10^{-7} cm^2 sec^{-1}) of Hb Portland 1 indicate a tetrameric structure with a molecular weight of 66,000. When the ultraviolet spectrum of Hb Portland 1 was compared for resolution of the tryptophan fine structure band just below 290 mμ, it demonstrated a fractional resolution (*8*) less than that of Hb Bart's but greater than that of Hb-F. Subunit hybridization studies of Hb Portland 1 with Hb-A (Fig. 2) showed four new species formed: two faster than either parent species and two slower. The faster components correspond to Hb Bart's (γ_4) and to Hb-H (β_4), the latter being so faint that it failed to photograph. The appearance of Hb Bart's indicates the existence of γ-chains in Hb Portland 1, while Hb-H presumably arises from the β-chains of Hb-A. The component that is slightly slower than Hb-A corresponds to Hb-F and is the result of recombination of α-chains from Hb-A and γ-chains from Hb Portland 1. The slowest component is thought to arise from recombination of the α-chains from Hb-A and some non-α-chains (x-chains) from Hb Portland 1; it is presumed to have the structure α_2x_2. The position of this slowest zone (α_2x_2) is near where Hb Gower 2 ($\alpha_2\epsilon_2$) migrates under these conditions (*9*). The structure γ_2x_2 is therefore proposed for Hb Portland 1.

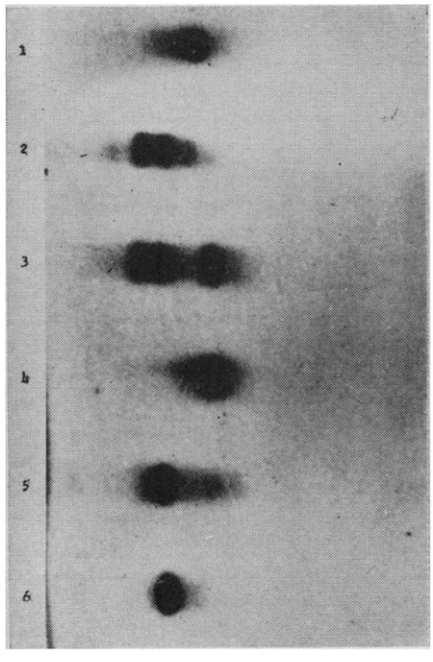

Fig. 2 (left). Starch-gel electrophoresis of recombination studies; Hb components read from left to right: (1) untreated Hb Portland 1, (2) recombinant of Hb Portland 1 + Hb-A (γ_2x_2, Hb-F, Hb-A, Hb Portland 1, Hb Bart's), (3) recombinants of Hb Bart's + Hb-A (Hb-F, Hb-A, Hb Bart's, Hb-H), (4) untreated Hb Bart's (5) untreated Hb-A + Hb Bart's + Hb-H, (6) untreated Hb-A.

The nature of the x chains was investigated by converting the pure Hb Portland 1 to globin by treatment with cold, acidified acetone, separation of the chains by countercurrent distribution, aminoethylation with ethylenimine, and digestion with trypsin, followed by columnar chromatography of the resulting peptides by Jones's methods (*10*).

These studies yielded nine unique peptides from the x-chain having amino acid compositions different from those of any tryptic peptides of the α, β, γ, or δ-chains; they indicate that the x-chain may be a new, previously undescribed hemoglobin chain. However, the appearance of a hemoglobin component having electrophoretic mobility similar to that of Hb Gower 2, following hybridization of Hb Portland 1 with Hb-A (see text above), suggests that the x-chains may be identical with the ϵ-chains. Later our studies of Hb Portland 1 will be reported in detail.

In every instance of known hemoglobins consisting of two different types of chains, such as Hb-A ($\alpha_2\beta_2$), Hb-F ($\alpha_2\gamma_2$), Hb-A_2 ($\alpha_2\delta_2$), and Hb Gower 2 ($\alpha_2\epsilon_2$), there is always an α-type pair of chains plus a non-α-type pair. Hemoglobin Portland 1 (γ_2x_2) represents the first occurrence in vivo of a hemoglobin having two unlike pairs of chains neither of which is of α-chains.

In a chromosomal triplication (*D*-trisomy), small amounts of Hb Gower 2 ($\alpha_2\epsilon_2$) found after birth (*9*) indicated persistence of the embryonic ϵ-chain. We believe that Hb Portland 1 similarly represents an embryonic hemoglobin that persisted after birth in relatively large amounts because of the patient's chromosomal abnormality. The large amount of Hb Bart's (γ_4) found in the propositus indicates deficiency in α-chain production and suggests that the appearance of Hb Portland 1 reflects the excess of γ- and x-chains. A recent report (*11*) of an embryonic hemoglobin showing some similarities to Hb Portland 1 in its electrophoretic behavior suggests the possibility that these two hemoglobins are identical.

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References and Notes

1. G. L. Capp, dissertation, Department of Biochemistry, University of Oregon Medical School, 1966.
2. F. Hecht, R. T. Jones, R. D. Koler, in preparation.
3. E. Havinga, *Proc. Nat. Acad. Sci. U.S.* **39**, 59 (1953).
4. O. Smithies, *Biochem. J.* **61**, 629 (1955).
5. D. W. Allen, W. A. Schroeder, J. Balog, *J. Amer. Chem. Soc.* **80**, 1628 (1958).
6. R. T. Jones and W. A. Schroeder, *J. Chromatog.* **10**, 421 (1963).
7. H. G. Kunkel and G. Wallenius, *Science* **122**, 288 (1955).
8. H. Drescher and W. Kunzer, *Klin. Wochschr.* **32**, 92 (1954).
9. E. R. Huehns, F. Hecht, J. V. Keil, A. G. Motulsky, *Proc. Nat. Acad. Sci. U.S.* **51**, 89 (1964).
10. R. T. Jones, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 297 (1964).
11. A. Kaltsoya, P. Fessas, A. Stavropoulos, *Science* **153**, 1417 (1966).
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