Sound) or not (Magueyes Island, Puerto Rico). Much greater variation in salinity occurs in bodies of water with restricted sea connections, as at Long Reef in Aransas Bay, Texas, Franklin City on Chincoteague Bay, Virginia, and at Crab Point in the Newport River, North Carolina. Stations located landward of the offshore barrier islands but close to ocean inlets are intermediate in their salinity variation (Radio Island, Beaufort Inlet, North Carolina; Pass Marianne in Mississippi Sound). The large temperature spread for Aransas Bay and Mississippi Sound (Fig. 1) is due to long water-sampling periods. Sampling periods longer than about 6 months at the Atlantic Coast localities would give misleading data as shell secretion is usually interrupted by the cold season.

The strontium concentration in the shells of *C. virginica* increases with increasing mean temperature of the sampling period, as shown in Fig. 2*A*. The dashed line, fitted by least squares, passes through all the observed ranges of the strontium values (*7*). The Caribbean species, *C. rhizophorae*, is somewhat off the trend displayed by *C. virginica*.

The concentration of magesium in the grown portions of *Crassostrea* shells also shows an increase with increasing temperature (Fig. 3*A*), although it is less well pronounced. In particular, the two samples from the Gulf Coast deviate from the more general trend.

From the analytical data, the coefficients of distribution of strontium and magnesium, respectively, between calcites and waters have been computed:

$$K'_{Sr}=\frac{(N_{SrCO_3}/N_{CaCO_3})_{cal}}{(m_{Sr^{++}}/m_{Ca^{++}})_{aq}}$$

and

$$K'_{Mg}=\frac{(N_{MgCO_3}/N_{CaCO_3})_{cal}}{(m_{Mg^{++}}/m_{Ca^{++}})_{aq}}$$

where K' is the distribution coefficient, N is the mole fraction of a component in calcite, and m is the molal concentration of the cation in the water. Plot of K'_{Sr} against temperature in Fig. 2*C* shows that the distribution coefficient changes with temperature rather than with the Sr^{++}/Ca^{++} ratio in solution. It may be noted that the high strontium concentration in the sample from Pass Marianne, Mississippi, is probably due to the higher value of the Sr^{++}/Ca^{++} ratio in the water (Fig. 2*B*), as the value of K'_{Sr} for that sample is fairly consistent with other values.

If the distribution of strontium between water and live shell of *C. virginica* is primarily a function of the water temperature and the ratio $m_{Sr^{++}}/m_{Ca^{++}}$, then it may be compared with an equilibrium distribution in an inorganic system. At 25°C the value of K'_{Sr} in the system strontian calcite–aqueous solution is about 0.14 ± 0.02 (*8*), which is close to $K'_{Sr} \cong 0.12$ for the *C. virginica* calcite at the same temperature. The dependence of K'_{Sr} in inorganic systems on temperature above and below 25°C is not yet completely understood (*9*), and consequently the correspondence between biogenic and inorganic calcites at other temperatures is not certain.

The distribution coefficient for magnesium (Fig. 3*C*) retains the spread of the magnesium concentrations in the samples. However, K'_{Mg} correlates positively with temperature ($r = .771$, significant at the .025 level). Concentrations in the range 0.5 to 1.5 mole percent $MgCO_3$ in calcitic shells (Fig. 3*A*) are lower than the calculated concentrations in calcite at equilibrium with solution in which $m_{Mg^{++}}/m_{Ca^{++}}$ is about 5, as in Fig. 2*B* (*10*). The nonequilibrium and a greater variation in the concentration of magnesium in shells may be due to its greater participation in the physiological functions of animals, in general, and marine invertebrates in particular (*11*). The samples in Fig. 3*A* which pronouncedly deviate from the general trend are from endemic populations in the Middle Oyster River, Great Bay, New Hampshire, and in Aransas Bay; the Pass Marianne sample is also of local stock. As opposed to these occurrences, many oysters have been artificially transported between the Middle and North Atlantic sections of the coast, which could have obliterated some of the original differences between the populations. The dependence of magnesium concentrations on the geographic location of the sample is compatible with the reported genetic differences between the temperature races of *C. virginica* (*12*).

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References and Notes

1. H. A. Lowenstam, in *The Earth Sciences*, T. W. Donnelly, Ed. (Univ. of Chicago Press, Chicago, 1963), p. 137; K. K. Turekian and R. L. Armstrong, *J. Marine Res. Sears Found. Marine Res.* **18**, 133 (1960); O. H. Pilkey and H. G. Goodel, *Limnol. Oceanogr.* **8**, 137 (1963); J. B. Rucker and J. W. Valentine, *Nature* **190**, 1099 (1961).
2. K. E. Chave, *J. Geol.* **60**, 190 (1952); **62**, 266 (1954).
3. R. C. Likins, E. G. Berry, A. S. Posner, *Ann. N.Y. Acad. Sci.* **109**, 269 (1963).
4. J. R. Goldsmith, D. L. Graf, O. I. Joensuu, *Geochim. Cosmochim. Acta* **7**, 220 (1955). In some Recent echinoid calcites, furnished by D. M. Raup, the concentrations of magnesium found by volumetric titration (A. Lerman) were consistently higher by about 10 percent than the concentrations found by x-ray diffraction (M. J. Rutherford).
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6. C. M. Yonge, *Oysters* (Collins, London, 1962).
7. The sample from the Middle Oyster River in Great Bay, N.H., was not included in the least-squares computation, as the temperature for that locality is not known; some measurements for summer and fall, 1941 and 1942, are about the mean 17° to 18°C, cited in C. F. Jackson, *A Biological Survey of Great Bay* (Univ. of New Hampshire, Biological Institute, Durham, 1944), pp. 8, 51–53.
8. H. D. Holland *et al.*, *J. Geol.* **72**, 36 (1964).
9. ———, *Geochim. Cosmochim. Acta* **28**, 1287 (1964); *U.S. At. Energy Comm. Res. Dev. Rept., Tech. Rept.* No. 6; *Ann. Rept.* July 1, 1964.
10. A. Lerman, *Geochim. Cosmochim. Acta* **29**, 977 (1965).
11. P. L. Altman and D. S. Dittmer, Eds., *Biology Data Book* (Federation of American Socs. for Experimental Biology, Washington, D.C., 1964), pp. 192–194; K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Molluses* (Academic Press, New York, 1964), vol. 1.
12. E. Mayr, *Animal Species and Evolution* (Harvard Univ. Press, Cambridge, Mass., 1963), p. 315.
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Immunoelectrophoresis Reveals Collagen Solubility in Human Serum

Abstract. *A fraction of human-serum proteins which contains hydroxyproline migrates, on electrophoresis, with the α_2-β-globulins. Immunoelectrophoresis with rabbit antiserum to soluble human collagen reveals an immunologically collagen-like protein in the human serum.*

Le Roy *et al.* (*1*) have shown that a protein containing hydroxyproline exists in human plasma. The protein, termed "hypro-protein," is presumed to be collagen or one of its precursors; it has been identified by Sephadex-gel filtration as either an

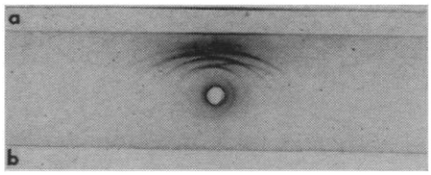

Fig. 1. Precipitin reaction in agar gel of human serum with antiserum to whole human serum (*a*) and antiserum to human collagen (*b*).

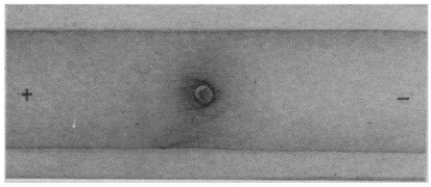

Fig. 2. Immunoelectrophoresis of human serum: precipitin reaction with antiserum to human collagen.

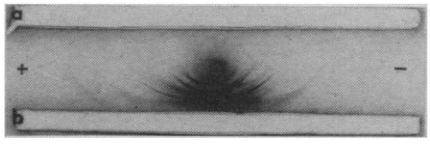

Fig. 3. Immunoelectrophoresis of human serum: precipitin reaction with (*a*) antiserum to collagen and (*b*) antiserum to whole human serum.

α_2-macroglobulin or an α- or β-lipoprotein. We (*2*) have found a hydroxyproline-bound protein localized in the α_2-β-globulins fraction; the fraction was characterized, after separation by column electrophoresis on acetolized cellulose (*3*). We have noted that antiserum to human serum gives a precipitin reaction with soluble human collagen; accordingly we have sought serum proteins showing the same antigenicity as collagen. We now report attempts to characterize such a serum fraction by immunoelectrophoresis.

We prepared antibodies from soluble human collagen; soluble collagen citrate was prepared by the method of Gallop and Seifter (*4*). Fragments of human skin, homogenized at 0°C in 0.5*M* sodium acetate in a VirTis-45 blendor were centrifuged at 5°C; the pellet was suspended in similar solution (at 4 ml per gram of tissue) for 18 hours and recentrifuged four times. The final pellet was washed four times by centrifugation from aqueous suspension. Soluble collagen was then extracted from the washed pellet by three successive 18-hour suspensions in 0.075*M* citrate buffer at *p*H 3.7 (at 4 ml per gram), each suspension being centrifuged for 1 hour at 18,000 rev/min. The final pellet was discarded. Soluble collagen was precipitated from the combined supernatants by dialysis against 0.02*M* disodium phosphate buffer. Purity of this soluble collagen was verified first by gel electrophoresis: it gave only one distinct zone. The amount of hydroxyproline in this preparation of soluble collagen was estimated to be 10.7 percent of the weight of protein.

Antiserums to collagen were prepared (*5*) by injecting, in divided doses, 2 ml of the following solution into the toe pads of each of three rabbits: equal parts of 1 percent collagen in citrate buffer and complete Freund's adjuvant. Four weeks after injection, the rabbits were bled, and the presence of antibodies to collagen was verified by the Ouchterlony technique. This antiserum gave a precipitin line when human serum was used as antigen (Fig. 1*b*); the line is comparable to the precipitin line produced by antiserum to whole human serum reacting with human serum used as antigen (Fig. 1*a*).

Electrophoretic localization in human serum of collagen-like antigen was tested by immunoelectrophoresis, according to Scheidegger's (*6*) modification of Grabar and William's procedure, with rabbit antiserum to human collagen. Study by microimmunoelectrophoresis suggests that in human serum there is a fraction that gives a precipitin reaction with specific antiserum to collagen (Fig. 2). At *p*H 8.4 this fraction has a slow rate of migration toward the anode and is situated near the starting zone.

We compared these results with those obtained by immunoelectrophoresis with rabbit antiserum to whole human serum (*7*). Antigenic, collagen-like, serum protein occurs in the α_2-β-lipoprotein zone (Fig. 3). This precipitin line is really due to collagen and not to β-lipoprotein, for it is not colored by Sudan Black BB, unlike lines given by the lipoprotein (*8*); and it is not removed after absorption of antiserum to collagen by purified β-lipoprotein prepared according to the Burstein technique (*9*). This fraction appears to be localized in the same zone as those fractions separated by cellulose-column electrophoresis.

These findings suggest that there is in human serum a collagen-like antigen that migrates electrophoretically with the α_2-β-protein fraction that contains hydroxyproline. Therefore we think that soluble collagen exists in human serum and presume that this soluble collagen is related to the collagen of connective tissue.

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References and Notes

1. E. C. Le Roy, A. Kaplan, S. Udenfriend, A. Sjoerdsma, *J. Biol. Chem.* **239**, 3350 (1964); H. Keyser, E. C. Le Roy, S. Udenfriend, A. Sjoerdsma, *Science* **142**, 1678 (1963).
2. J. Frey, *Biochim. Biophys. Acta*, in press.
3. J. Enselme, J. Tigaud, J. Frey, *Bull. Soc. Chim. Biol.* **41**, 1373 (1959).
4. P. M. Gallop and S. Seifter, *Methods Enzymol.* **6**, 635 (1963).
5. F. O. Schmitt *et al.*, *Proc. Nat. Acad. Sci. U.S.* **51**, 493 (1964).
6. J. J. Scheidegger, *Intern. Arch. Allergy Appl. Immunol.* **7**, 103 (1955).
7. Mixtures of antiserum to human serum, No. 223 and 306, from Institut Pasteur, Paris.
8. J. Uriel and P. Grabar, *Bull. Soc. Chim. Biol.* **38**, 1253 (1956).
9. M. Burstein and J. Samaille, *J. Physiol.* **49**, 83 (1957).

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Uptake as a Determinant of Methotrexate Response in Mouse Leukemias

Abstract. *Methotrexate-promoted survival of mice bearing different transplantable leukemias was compared with uptake of the drug by leukemia cells in vitro. A high degree of correlation* ($r^2 = 0.88$) *showed that uptake may be a major determinant of the antitumor activity of methotrexate against transplantable mouse leukemias.*

Methotrexate is a chemotherapeutic agent which initially controls certain mouse and human leukemias. This drug is tightly bound by the enzyme dihydrofolate reductase (*1*). The resulting inhibition of this enzyme blocks reduction of folate and dihydrofolate to tetrahydrofolate, and this leads to interference with one-carbon metabolism, causing death of growing cells (*2*). The effectiveness of methotrexate as an antileukemic agent is limited by the eventual development of resistance (*3*).

Figure 1 shows the relative sensitivity of ten transplantable mouse leukemias to methotrexate as measured by drug-promoted survival of tumor-bearing animals (*4*) and the corresponding uptake of the drug by each cell line in vitro. A significant correlation ($r^2 = 0.88$) exists between these two variables, showing that the drug uptake by each cell line is an important determinant of survival of tumor-bearing animals.