336 μg of arginine per milliliter of culture has resulted in greater early stimulation of cellular proliferation, although the final cell populations did not approach that achieved with the 42 μg/ml level. No manifestations of toxicity were apparent at the higher concentrations.

Addition of arginine has been found to be useful for the maintenance of strain HeLa or Westwood's ERK/KD cell line (*6*). In addition, arginine has been used effectively as a supplement in a serum-free medium employed for maintenance of the ERK/KD cells in submerged culture (20-liter) during the experimental production of viral antigens (*7*). In some instances, active cellular proliferation has been maintained for periods up to 20 days through the addition of arginine and new media.

Several possibilities relating to the mechanisms of this action of arginine may be mentioned, such as one similar to the mechanism described for the protection, by arginine, of rats against ammonia toxicity (*8*). Again, arginine may be incorporated directly into tissue protein, as was demonstrated by Bloch (*9*). We have found that citrulline (but not ornithine) will operate in the same fashion and to the same degree of efficacy as arginine in cultures of L cells. The mechanism of action of arginine in cultures of mammalian cells has not been determined as yet, and further comment on the subject must await more detailed studies.

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Oxygen Equilibrium of Phascolosoma agassizii Hemerythrin

Hemerythrin is the respiratory pigment of the phylum Sipunculida, whose members are small-to-medium-sized worms frequently found buried in an oxygen-deficient substratum. Hemerythrin is reddish-purple when oxygenated, colorless when deoxygenated; this is in contrast to the other two iron-containing respiratory pigments, hemoglobin and chlorocruorin. Hemocyanin, the oxygen-binding copper protein of arthropods and molluscs, resembles hemerythrin in being colorless when deoxygenated. On the basis of chemical and thermodynamic evidence, Klotz and Klotz (*1*) have concluded that oxygenation of hemerythrin and hemocyanin differs from that of heme respiratory pigments in that the metal undergoes a valence change upon the reversible combination with O_2. Klotz and Klotz have reported that the heat of oxygenation of *Golfingia* [formerly *Phascolosoma* (*2*)] *gouldii* is about twice that of hemoglobin and have utilized this fact as evidence for the acceptance of two electrons by the oxygen molecule in oxyhemerythrin—in contrast to oxyhemoglobin, where the ligand does not gain electrons.

However, two other workers have observed that the effect of temperature on the oxygen equilibrium of the hemerythrin of *Sipunculus nudus* (*3*) and of a species of *Golfingia* (*4*) was very similar to its effect on the oxygen-hemoglobin equilibrium. This, coupled with the small amount of published information on the oxygen-hemerythrin equilibrium, has warranted a study of the hemerythrin of the "peanut worm," *Phascolosoma* (*Physcosoma*) *agassizii* Keferstein (*5*).

Hemerythrin was prepared by distilled water hemolysis of clotted coelomic cells, followed by centrifugation and filtration. Forty specimens of this small sipunculid yield approximately 1 ml of a 3 percent hemerythrin solution. Oxygen dissociation curves were determined by spectrophotometric analysis ($\lambda = 580$ to 600 mμ) of the mixture of "reduced" and oxygenated hemerythrin formed upon equilibration with known partial pressures of oxygen. The partial pressure of oxygen was varied by injection of known quantities of air into a confined space with the respiratory pigment, as in the technique of Riggs (*6*).

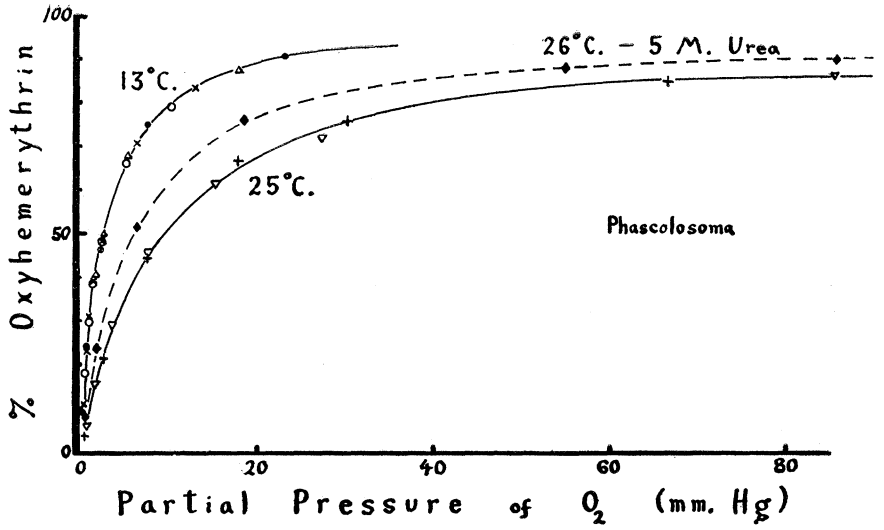

Fig. 1. Oxygen equilibrium of *Phascolosoma agassizii* hemerythrin. Two percent hemerythrin in potassium phosphate buffer, $\Gamma/2 = 0.2$. Solid lines: no urea present; experiments at 13°C performed at *p*H's of 7.90, 7.23, 6.79, and 5.75; experiments at 25°C performed at *p*H's of 7.74 and 7.27. Dashed line: 5*M* urea present; experiment performed at 26°C and a *p*H of 8.08.

The result of experiments on the effect of *p*H, temperature, and urea on the oxygen-hemerythrin equilibrium is shown in Fig. 1. Since the position of the oxygen dissociation curve is invariant to *p*H change, this particular hemerythrin lacks a Bohr effect, as do other sipunculid hemerythrins (*3*, *4*). Although a slightly sigmoid oxygen dissociation curve has been observed for *Sipunculus* hemerythrin (*3*), *Phascolosoma agassizii* hemerythrin possesses a hyperbolic curve.

From the data (Fig. 1) on *Phascolosoma agassizii* hemerythrin at two different temperatures, it is possible to calculate (*7*) a heat of oxygenation ($\Delta H°$) in terms of a standard state of 1-atm pressure of dissolved oxygen gas; the following integrated form of the van't Hoff equation was used:

$$\ln\left[\frac{p_{50}(T_1)}{p_{50}(T_2)}\right] = \frac{\Delta H°}{R}\left[\frac{T_2 - T_1}{T_1 T_2}\right],$$

where $p_{50}(T)$ represents the partial pressure of O_2 (in atmospheres) at which, at the specified temperature, there is exactly 50 percent of the respiratory pigment in an oxygenated condition. When the oxygen dissociation curve is hyperbolic, the equilibrium constant is the reciprocal of $p_{50}(T)$. For this particular hemerythrin, a value of $\Delta H° = -17$ kcal was observed; this is

closer to the recently observed value of −18 kcal for *Golfingia gouldii* hemerythrin (*2*) than to corresponding values of earlier workers (*3, 4*), which are approximately −10 kcal. The most recently observed values for hemoglobin are significantly lower: −8.2 kcal for sheep hemoglobin (*8*) and −8.4 kcal for *Cucumaria miniata* hemoglobin (*9*). Preliminary experiments with the coelomic hemerythrin of *Dendrostomum zostericolum* indicate that the heat of oxygenation is −20 kcal. Hence, this aspect of the trend in values of $\Delta H°$ predicted by Klotz and Klotz (*2*) has been confirmed.

Also in terms of a standard state of 1 atm of dissolved oxygen gas, an entropy of oxygenation can be calculated (*7*) from the relations:

$$\Delta F° = -RT \ln K = RT \ln p_{50}$$

and

$$\Delta S° = -\frac{\partial(\Delta F°)}{\partial T}$$

For *Phascolosoma agassizii* hemerythrin $\Delta S° = -46$ cal/deg, as compared with values approximating −10 to −15 cal/deg for various hemoglobins (*10, 11*). For hemoglobin these negative entropies, together with other biochemical evidence have been interpreted (*11, 12*) as indicating that, upon oxygenation of "reduced" hemoglobin, a definite configurational change in the protein moiety occurs, oxyhemoglobin being the more "ordered" form of the protein. Such an explanation would be plausible for hemerythrin, particularly in that such an entropy change during oxygenation cannot represent changes in the state of aggregation of solvent molecules about proton-affine centers in the protein moiety —for hemerythrin lacks a Bohr effect. That the oxygen equilibrium of hemerythrin as with hemoglobin (*11*) depends on the configuration of the protein moiety is also shown by the increase in oxygen affinity upon addition of urea (Fig. 1).

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Separation of Human Plasma Protein Concentrate with Insulin Activity

Insulinlike activity of human plasma has been identified in plasma fraction II + III as obtained by the cold-ethanol method and in plasma globulin precipitate obtained by equilibrating plasma with 50 m*M* zinc (*1*). Identical fractions prepared from resin-collected plasma were inactive. The suggestion that cationic exchange resins (Dowex-50, IRC-50) employed for blood collection remove the insulin activity was verified when an attempt was made to elute the insulinlike activity from resins. In these experiments it was calculated that 40 percent of the total plasma activity was recovered from the cationic exchange resins with 0.3*M* citric acid, at *p*H 3.0 (*2*).

The percentage of recovery has been increased by treating the resins after blood collection with 1 resin volume of 0.2*M* citric acid at 2°C for 72 hours (*3*). The resins were washed two times, before elution, with cold distilled water to remove the red blood cells and the contaminating proteins and, finally, were washed once with 0.15*M* sodium chloride. After dialysis of the eluate for 36 hours against four to five volumes of distilled water at 2°C, the solution was dried from the frozen state, and the residue was dissolved in the smallest possible volume of cold water. Dialysis followed, for 48 hours, against 0.15*M* sodium chloride at 2°C.

Biological tests for insulin activity of the resin eluates were performed by using, respectively, hypophysectomized-alloxanized rats (*4*); epididymal fat tissue (*5*); and rat diaphragm (*6*). The recovery of the original plasma insulin activity from the resin was indicated variously at from 40 to 80 percent by the above methods.

Eluates from resins representing the collection of 30 to 40 lit of human plasma were pooled, and the volume was reduced to 2 ml. Paper electrophoresis was performed on the Spinco model R, series D, paper electrophoresis cell, at 2.5 ma for 18 hours, with Veronal buffer, *p*H 8.6, ionic strength 0.1, and S and S 2043A-mgl paper strips, 3.0 by 30.6 cm. Insulin solution (0.018 ml) was applied on the paper. A plasma control was run at the same time by applying 0.006 ml of plasma. The paper electrophoresis indicated the presence in the eluate of an α_1-globulin and a γ-globulin when compared with the plasma control (Fig. 1). A portion of the protein in the γ-globulin area may be denatured protein, as might be expected from acid treatment for 72 hours.

In another study, 0.2 ml of the insulin solution was applied on a 4 by 31 cm paper strip (S and S 470); this was run at 40 ma for 16 hours at 2°C, with a phosphate buffer, *p*H 7.6, ionic strength 0.1. A plasma control was also run in the same machine. The control strip carrying the plasma was then developed, and the paper strip carrying the insulin solution was cut in five sections: the albumin, the α_1-globulin, the α_2-globulin, the β-globulins, and the γ-globulin. Elution followed, with 0.15*M* sodium chloride, and the eluates were tested for insulin activity (*5*). The α_1-globulin and the albumin section possessed ininsulin activity. The rest of the eluates were found to be inactive. The activity present in the albumin section may be due to the presence of some α_1-globulin, which is difficult to separate from albumin. There was a strong suggestion from the bioassay that the γ-globulin section had an inhibitory effect. The protein concentration of the final insulin concentrate was more than 1.5 million times that of the plasma.

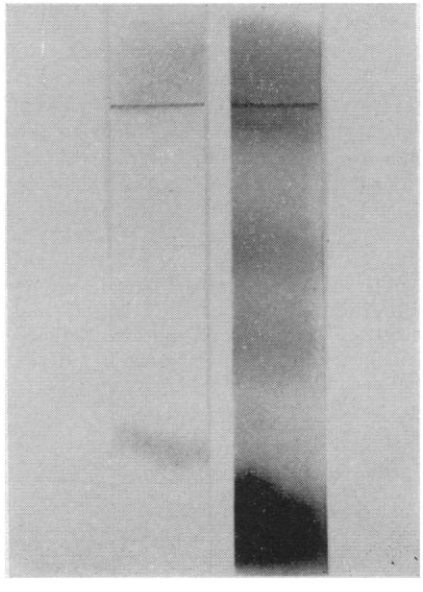

Fig. 1. Paper electrophoresis of the insulin concentrate (left) and plasma control (right). The protein concentration of the insulin concentrate is more than 1.5 million times that of the original plasma.