immune phenomena, and lymphoproliferative disorders in several models, including New Zealand Black mice (*3, 14*) and chronic graft versus host disease (*3*), suggests that the same relationships may exist in man. In all of these situations, the common thread may be a virus-induced autoaggressive reaction that may or may not progress to neoplasia depending on the interrelationships between clonal proliferation of autoimmune cells and antiviral and antitumor immunity.

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Chemical Facilitation of Thermal Conduction in Physiological Systems

Abstract. *Experimental evidence supports the concept that solutes capable of reversible chemical reaction can passively facilitate thermal conduction within their solutions. Myoglobin and bicarbonate are suggested as energy carriers in muscle, having the combined capacity for conveying all waste metabolic heat produced under normal physiological conditions. The concept is extended to convective heat transfer in vivo; this implicates hemoglobin.*

There are a number of physiological systems in which the transmission of a gas in solution can be passively facilitated by the diffusion of a carrier for which it has a reversible chemical affinity, for example, oxygen in combination with myoglobin (*1*). A similar regenerative process is proposed here for the conduction of heat.

Let us consider a closed system in which a particular substance can exist in two chemical states, A and B, which have different enthalpies, H, and are readily interconverted according to the rapid reversible reaction:

$$\underset{(1-x)}{\mathrm{A}} \rightleftarrows \underset{(x)}{\mathrm{B}} + \text{heat}\ (-\Delta H) \quad (1)$$

where the position of equilibrium, and hence the degree of conversion, x, are determined at any point by the local temperature, T, according to the Van't Hoff isochore.

If the system is macroscopically static, and opposite sides are maintained at different temperatures, T_1 and T_2 (Fig. 1), then the substance must act as a passive energy carrier if it is capable of translational movement within the medium. Molecules moving up the temperature gradient will tend to assume form A, with absorption of heat,

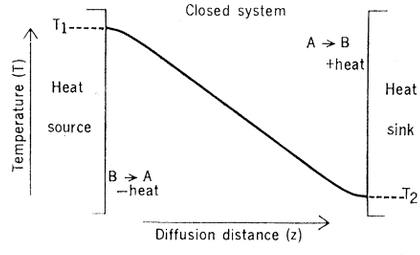

Fig. 1. A closed system with opposite sides maintained at different temperatures, T_1 and T_2.

while those whose random thermal motions take them to cooler regions will tend to revert to B with release of heat. Thus the transfer of energy from the region of the source, T_1, to that of the sink, T_2, can be effected by the diffusion of carrier molecules which can also exchange heat transmitted by simple thermal conduction. An analysis of the system in Fig. 1 has been made (*2*) and gives expressions exactly analogous to those for facilitated diffusion (*3*). For the ideal case of local chemical equilibration, these reduce to a simple form giving the overall steady-state heat flux ($\dot{Q}$) as:

$$\dot{Q}\,\bar{z} = K(T_1 - T_2) + DC(\Delta H)(x_2 - x_1) \quad (2)$$

where $\bar{z}$ is the effective mean path length; K is the thermal conductivity without carrier; D is the mean diffusion coefficient of the carrier whose molar concentration is C; and x_1 and x_2 are the degrees of conversion at temperatures T_1 and T_2 respectively. The first term on the right side in this equation represents simple conduction, while the second represents chemical facilitation.

As a simple test of this thermal carrier hypothesis, alternating current was used to generate heat at a fixed rate in a thermistor whose temperature was recorded by a d-c circuit. The thermistor did not reach as high a temperature immersed in hemolyzed human blood at $P_{O_2} = 53$ mm-Hg and $P_{CO_2} = 38$ mm-Hg as it did when the hemoglobin was "poisoned" by the substitution of 1 percent CO for N_2 in the equilibration gas at the same oxygen and carbon dioxide tensions. In another series of experiments, the temperature was slightly higher for normal saline than for isotonic bicarbonate solution at $P_{CO_2} = 38$ mm-Hg.

Since convection is dependent upon conduction, whichever of these processes was primarily responsible for the thermistor cooling, it is difficult to invoke any interpretation of these findings other than an increase in the thermal conductivity. At least, this holds when one solute is present under conditions conducive to its reversible chemical transformation over the prevailing temperature gradients, namely, Hb at $P_{O_2} = 53$ mm-Hg and HCO_3^- at $P_{CO_2} = 38$ mm-Hg according to the reactions:

$$\mathrm{Hb} + \mathrm{O_2} \rightleftarrows \mathrm{HbO_2} + 18\ \text{kcals} \quad (3)$$

$$\mathrm{H^+} + \mathrm{HCO_3^-} \rightleftarrows \mathrm{H_2CO_3} + 2\ \text{kcals} \quad (4)$$

The above findings are consistent with the unusually high heat transfer rates in the gaseous phase that have been

found for N_2O_4 which can undergo reversible thermal dissociation (*4*).

In looking for practical systems in which the foregoing concepts may apply, it is clear that the basic requirements for chemical facilitation of both forms of heat transfer are present within the muscle fiber. Brownian motions necessary for diffusional augmentation of thermal conduction have been observed (*5*) within biological cells, despite the gel-like consistency of cytoplasm, while the occurrence (*6*) of countercurrent cytoplasmic streaming is suggestive of convective heat transfer.

Moreover, the experiment with hemoglobin solution indicates that myoglobin should also provide facilitation of heat transfer—in fact more so by virtue of its lower molecular weight (approximately ¼) and higher diffusion coefficient. The equation for myoglobin is the following:

$$Mb + O_2 \rightleftarrows MbO_2 + 13.5 \text{ kcal} \quad (5)$$

The general system outlined in Fig. 1 can be applied to the muscle fiber, with the mitochondria as the heat sources, T_1, and the cell wall as the heat sink, T_2. The potential heat carriers include both myoglobin and bicarbonate. However, when typical values for muscle fiber are substituted in the expression for a closed system (Eq. 2), the contribution of chemical facilitation, $DC(\Delta H)(x_2 - x_1)$, is small compared to the contribution of simple thermal conduction, $K(T_1 - T_2)$, because of the low values for D in liquids relative to K. This is so unless $(T_1 - T_2)$ is small relative to $(x_2 - x_1)$. This situation is unlikely in a system totally closed with respect to mass because the positions of chemical equilibration must be close in the vicinities of heat source and sink when their temperatures are similar—unless the enthalpy change, ΔH, is particularly high.

However, while many physiological systems may be closed with respect to the major or rate limiting energy carrier, such as myoglobin in cytoplasm, they can be open with respect to any other reagent involved. Moreover, if a nonuniform distribution of this other reagent is maintained by metabolism, it is possible to have a significant value for $(x_2 - x_1)$ while ΔT is very small, zero, or even negative.

The intriguing feature of the gradients of the respiratory gases involved is their displacement of the chemical equilibria for both O_2 and CO_2 reactions (Eqs. 3, 4, and 5) toward the right at the blood boundary and toward the left at the mitochondria. Thus the transmission of O_2 and CO_2 occurs in directions which enable both myoglobin and bicarbonate to facilitate the transfer of waste metabolic heat in the desired direction (Fig. 2). Moreover, the CO_2 tension range in the cell corresponds to a significant change in bicarbonate conversion (Eq. 4) according to equilibrium constants, while the O_2 tension range (*1*) is almost ideal, giving an 80 to 95 percent change in the dissociation of oxymyoglobin (*7*). These ranges are also particularly conducive to facilitated diffusion of O_2 by myoglobin (*1*) and CO_2 as bicarbonate (*8*).

Under these nearly optimal conditions, it is interesting to reassess the magnitude of facilitation in muscle fiber considered as a semi-closed system. The displacement of carrier conversion by the permanent gas gradients (Fig. 2) must elevate $(x_2 - x_1)$ relative to $(T_1 - T_2)$, and hence the magnitude of chemical facilitation relative to simple thermal conduction (Eq. 2). A similar argument also applies to heat transfer effected by any convection.

The above comparison can be extended to isothermal conditions where $T_1 = T_2$. Here all contributions from physical transmission pathways are eliminated and only the facilitated term remains significant in Eq. 2. The substitution (*2*) of typical values (*1*, *7*) provides estimates of the heat carrying capacities, $\dot{Q}\bar{z}$, of 2.4×10^{-8} cal cm^{-1} sec^{-1} for the bicarbonate and 5.2×10^{-9} cal cm^{-1} sec^{-1} for the myoglobin normally found in a skeletal muscle fiber. Allowing a mean path length, $\bar{z}$, of 10 μm, these figures imply a total capability for heat transfer by chemically facilitated conduction of 0.029 cal cm^{-3} sec^{-1}. This figure just exceeds a maximal heat production of 0.025 cal cm^{-3} sec^{-1} estimated from a value of 77 ml of O_2 per second per kilogram of total body weight for elite middle distance runners during extreme exercise (*9*). Thus there would appear to be just enough carrier present, in the form of myoglobin and bicarbonate, to transfer all waste metabolic heat in the absence of any temperature differential between the mitochondria and the cell wall. At least, there is the chemical capability whatever the contribution of physical processes to total heat transmission is in reality.

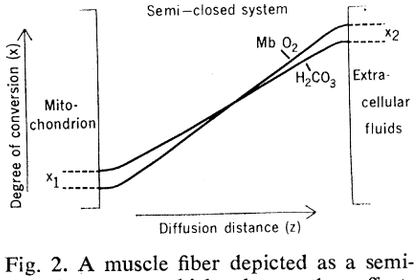

Fig. 2. A muscle fiber depicted as a semi-closed system which shows the effects of permanent O_2 and H_2CO_3 gradients, both maintained by metabolism. These gradients displace carrier conversions, x, in the directions indicated, so that both reaction systems tend to absorb heat at the mitochondria and to release it in the region of the cell wall. At the mitochondria, the reactions are the following: $MbO_2 \rightarrow Mb + O_2 - 13.5$ kcal and $H_2CO_3 \rightarrow H^+ + HCO_3^- - 2$ kcal. In the region of the cell wall, the reactions are these: $Mb + O_2 \rightarrow MbO_2 + 13.5$ kcal and $H^+ + HCO_3^- \rightarrow H_2CO_3 + 2$ kcal.

The above calculations have important physiological implications because they provide another reason for the presence of myoglobin and bicarbonate in the particular concentrations in which they are found in the body. This suggests a role for these substances as heat carriers, in addition to gas carriers, in maintaining metabolic efficiency at high work rates. In mammals, this efficiency is known (*10*) to decrease markedly with small rises in temperature of the mitochondria.

In addition to facilitating energy and mass transfer, chemical carriers of rapidly reversible affinity might also provide an instantaneous source of heat and so add to the known complexity (*11*) of muscle thermodynamics.

While improved heat transfer seems an obvious advantage in mammals, the reverse argument can be applied to creatures living in particularly cold environments yet possessing comparatively poor thermal insulation, for example, the icefish of the Antarctic. Any facilitation of thermal conduction would be detrimental. Hence it is tempting to speculate that, in order to maintain body temperature above ambient as certain fish attempt to do (*12*), it proved more efficient for the icefish to lose its heme pigments during its probable evolution from chaenichthids despite their advantages (*1*) for carrying and storing oxygen.

While the preceding discussion has considered only cellular conduction, the exchange of oxygen between hemoglobin and myoglobin enables an appreciable proportion of the waste metabolic energy to be transmitted to blood in a chemical form for subsequent release as heat at the lungs. This has a number of implications, for instance, in hyperoxia, to the extent that when Hb in ve-

nous blood is saturated with O_2 there can be no chemical removal of heat from tissue by blood. Heat can then accumulate in a tissue, such as the brain, which is not readily able to increase its perfusion rate. Hence it is interesting that neurological oxygen toxicity becomes manifest at about the point of venous saturation of hemoglobin and that it is particularly sensitive to temperature—a man with fever readily convulses in hyperbaric oxygen (*13*).

Finally, this discussion has merely touched upon a few of the most obvious implications of this novel concept of chemical facilitation of heat transfer in physiological systems.

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Collagen Synthesis: Localization of Prolyl Hydroxylase in Tendon Cells Detected with Ferritin-Labeled Antibodies

Abstract. *An improved procedure was employed for linking ferritin to antibodies against prolyl hydroxylase, the enzyme that synthesizes the hydroxyproline in collagen. By electron microscopy, the enzyme was then found to be localized in cisternae of the rough endoplasmic reticulum of embryonic tendon cells; this indicates that hydroxylation of proline occurs while newly synthesized polypeptides are fed into the cisternae.*

The collagen molecule is synthesized by a series of steps that include (i) assembly of amino acids into each of the three polypeptide chains of the molecule, (ii) hydroxylation of some of the prolyl and lysyl residues in the polypeptide chains, and (iii) glycosylation of some of the hydroxylated lysyl residues before the molecule is secreted [for review, see (*1*)]. Some of the hydroxylations of proline and lysine can occur while the nascent chains are still being assembled on ribosomes, but these residues can also be hydroxylated after chain completion. If the prolyl and lysyl hydroxylases are inhibited, freshly isolated connective tissues or cells synthesize and accumulate an unhydroxylated form of collagen known as protocollagen (*1, 2*). If inhibition of the hydroxylases is reversed under appropriate conditions, the accumulated protocollagen is hydroxylated and secreted. Although these and related observations demonstrate that the molecule must be extensively modified before it is ready for secretion, relatively little is known about which cellular organelles are involved. For example, although collagen-like aggregates have been observed in Golgi vacuoles (*3*), it has not been established that collagen is synthesized and secreted via the rough endoplasmic reticulum and Golgi complex (*4*), as shown for several other proteins synthesized for "export" (*5*). In the experiments reported here, improved procedures with ferritin-labeled antibodies were used to locate prolyl hydroxylase, the enzyme that synthesizes hydroxyproline in collagen.

Prolyl hydroxylase was purified from chick embryos by means of an affinity column procedure (*6*), and antibodies were prepared in rabbits (*7*). The antibodies were purified by immunoadsorption on a column containing pure prolyl hydroxylase covalently bound to 1 percent agarose (Bio-Rad, A-150m) (*8*). The column was eluted with 3*M* sodium thiocyanate in 0.01*M* sodium phosphate, *p*H 6.0 (*9*), and the purified antibody was linked to ferritin with glutaraldehyde by an improved procedure (*10*). Ferritin (Polysciences; six times recrystallized) was activated by incubation (3.3 mg/ml) in a solution of 1.7*M* glutaraldehyde (Baker) in 0.1*M* sodium phosphate, *p*H 7.3, at room temperature for 30 minutes. The activated ferritin was separated from excess glutaraldehyde by gel filtration on a Sephadex G-25 (Pharmacia) column equilibrated and eluted with 0.1*M* sodium phosphate buffer, *p*H 7.3. Purified antibody (1 mg) was then added to 2 mg of activated ferritin in 11 ml of the phosphate buffer. After 26 hours at 4°C, the product was isolated by gel filtration on a 6 percent agarose column (Bio-Rad, A-5m) equilibrated and eluted with 0.1*M* tris(hydroxymethyl)-aminomethane (tris) hydrochloride buffer, *p*H 7.5, at 4°C. The method was an improvement over earlier procedures (*11*) in that the yields were more than tenfold greater, the product was free of unconjugated antibody, the antibody retained most of its immuno-

Treatment	Enzyme protein per 10^8 cells	
	Micrograms	Percent of total
Homogenate (nonfixed)	2.7	100
Supernatant 1	0.25	9
Supernatant 2	0.57	21
Cell fraction	1.36	50

Table 1. Determination of prolyl hydroxylase in fixed cell preparations by passive hemagglutination inhibition. Tendon cells (5×10^8) were incubated in 66 ml of modified Krebs medium at 37°C for 3 hours (*2*). The sample was divided in half, and the cells were separated from the medium by centrifugation. The cells from half of the sample were suspended in 2.5 ml of 0.1*M* sodium phosphate buffer, *p*H 7.3, and homogenized with 60 strokes in a Teflon and glass homogenizer at 1740 rev/min with a constant-torque motor (Schwaben Präzision, Nördlingen, Germany). The homogenate was used to measure total enzyme antigen. The cells from the other half of the sample were fixed by incubation for 1 hour at 4°C in 2.5 ml of a solution containing 1 percent formaldehyde (Baker), 0.06*M* sodium phosphate buffer, *p*H 7.3, and 0.14*M* sucrose. The cells were removed by centrifugation and washed with 2.5 ml of 0.1*M* sodium phosphate buffer, *p*H 7.3. The wash was combined with the fixing solution; dialyzed against 500 ml of buffer containing 0.2*M* NaCl, 0.1*M* glycine, and 0.01*M* tris-HCl, *p*H 7.8, at 4°C; and concentrated to 0.5 ml in an Amicon ultrafiltration cell with a PM-30 membrane. This sample was supernatant 1. The cell pellet was suspended in 2.5 ml of 0.1*M* sodium phosphate buffer, homogenized with 30 strokes as described above, and centrifuged at 20,000*g* for 20 minutes. The supernatant and pellet were supernatant 2 and the cell fraction, respectively. Enzyme protein was measured by a passive hemagglutination inhibition technique as described by Eskeland *et al.* (*15*), except that 0.1 percent bovine serum albumin was used instead of 0.1 percent gelatin. Formalin-treated sheep blood cells were coated with prolyl hydroxylase (*7*) and a standard curve with purified prolyl hydroxylase was prepared (*6*).