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HEMOGLOBIN, GLUCOSE, OXYGEN AND WATER IN THE ERYTHROCYTE

A Concept of Biological Magnitudes, Based upon Molecular Dimensions

By Dr. DAVID L. DRABKIN

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA

THE purpose of this communication is to direct the attention of biological workers to the illumination which may be afforded in many problems by expressing, when possible, biochemical relationships on a molecular basis.

The use of empirical "units" in the literature of the vitamins and hormones is justified in the early stages of investigation before precise quantities can be employed. However, in many instances the usage of "units" of this or that has continued long after the chemistry of the therapeutic or prophylactic agent has been established. The persistence of such empiricism is not only irksome, but serves to obstruct quantitative thinking and often delays proper interpretation and formation of useful concepts. With the enlarging interest in the architectural chemistry of cells and the dynamics of cellular metabolism, the realization appears inevitable that quantitation even on such a basis as "gm or mg per 100 ml" may have but limited usefulness. This is more obvious in a consideration of the complex equilibria which are the chemical mechanisms of cellular work and energy supply.

My attention was drawn forcibly to the advantages and desirability of calculating the concentration of cellular elements in terms of molecular populations

in the course of a study of the effect of fluoride and iodoacetate upon the disappearance of methemoglobin (oxidized hemoglobin), MHb, from blood on standing. In this *in vitro* system whole blood (with approximately 50 per cent. of the hemoglobin converted intracellularly to MHb) was employed in the presence of glucose, added to make a total concentration of about 300 mg per 100 ml. Upon incubation of such blood samples at 38° C in the presence of air, MHb reverted to oxyhemoglobin, and there was a parallel disappearance (glycolysis) of glucose. The addition of fluoride or iodoacetate inhibited both the glycolysis and the reversion of MHb. The inhibition owing to fluoride appeared to be partially overcome by the addition of pyruvate. In the interpretation of the significance of these findings the question arose as to the probability that a similar mechanism could function *in vivo*. The concentration of MHb in circulating blood is normally very low,[1] and when this pigment is produced in the organism in abnormal amounts it is removed rapidly from the circulation.[2] Is the interrelationship of glycolysis and MHb reduction physiological in view of the relative concentrations of glucose and hemoglobin in the erythrocyte? Our analytical values for adult human blood agree with the literature, and are for hemoglobin very high, approximately 34 gm per 100 ml (hematocrit packed volume) of red blood cells, and for glucose quite low, of the order of 0.075 gm per 100 ml of cells.[3] Expressed in these usual terms, the values apparently emphasize the overwhelming concentration of hemoglobin. But it will be seen that when calculations are made upon the basis of their respective molecular populations within the red blood cell, the concentrations of glucose and hemoglobin become virtually identical. It will also be apparent that the molecular population of even so "simple" a cell as the mammalian erythrocyte is closely packed. Hence, the interaction of these components in their cellular environment is rendered more probable.

Several examples of calculations follow. These were chosen in the fields most closely related to the writer's interests, and represent cases where definite data are available to make such calculations with some degree of confidence. No doubt many other examples can be found to supply equally instructive appraisals of metabolic phenomena within cells and tissues. It may be pointed out that the relationships to be discussed are implicit in values for concentration in molar terms, moles, M, per chosen volume.

[1] D. L. Drabkin and C. F. Schmidt, *Jour. Biol. Chem.*, 157: 69, 1945.

[2] W. W. Cox and W. B. Wendel, *Jour. Biol. Chem.*, 143: 331, 1942.

[3] M. Somogyi, *Jour. Biol. Chem.*, 78: 117, 1928.

1. *Calculation of Molecular Population of Hemoglobin in an Erythrocyte*

(*a*) Corpuscular volume (volume of individual cell in packed state): Erythrocyte count = 5×10^6 per mm^3.

Hematocrit value = 45 per cent., or 45 ml per 100 ml of blood.

Packed cell count = 5×10^6 per 0.43 mm^3 or per 4.5×10^8 μ^3 (since 1 $mm^3 = 10^9$ μ^3).

Therefore, volume of 1 cell = $4.5 \times 10^8 / 5 \times 10^6 = 90$ μ^3 (90 cubic microns).

(*b*) Cellular hemoglobin concentration = 340 gm per 1,000 ml or per 10^{15} μ^3 (since 1 ml = 1 $cm^3 = 10^3$ $mm^3 = 10^{12}$ μ^3). Hence, per cell of 90 μ^3 volume the hemoglobin concentration is 30.6×10^{-12} gm, or 30.6 micro-micrograms ($340 \times 90/10^{15}$).

Since the molecular weight of hemoglobin is 66,700 (on the basis of 0.335 per cent. of Fe and 4 atoms of Fe), the molar concentration of the pigment = $340/66{,}700 = 5.1 \times 10^{-3}$ M per 10^{15} μ^3.

Therefore, since 66,700 gm of hemoglobin contain 6.02×10^{23} molecules (Avogadro's number—number of molecules per gram molecular weight), the molecular population in hemoglobin of a single red blood cell with a volume of 90 $\mu^3 = 5.1 \times 10^{-3} \times 6.02 \times 10^{23} \times 90/10^{15} = 2.76 \times 10^8$, or approximately 300,000,000 molecules of hemoglobin per cell. In this calculation the molecular weight is that of the non-hydrated protein. According to x-ray measurements,[4] wet hemoglobin has a molecular magnitude of approximately 132,000. If the latter value were employed the original concentration of hemoglobin in gm per liter of cells would have been taken as 673 gm (from $340 \times 132{,}000/66{,}700$). Hence, the same final result would be yielded in the calculation of molecular population. However, the hydration of the molecule may have to be considered in a calculation of the "space occupied" by the hemoglobin in the cell (see 3*c*).

2. *Calculation of Molecular Population of Glucose in a Red Blood Cell*

Cellular glucose concentration = 0.75 gm per 1,000 ml or per 10^{15} μ^3.

Hence, the glucose concentration per corpuscle of 90 μ^3 volume = $0.75 \times 90/10^{15}$ gm = 6.75×10^{-14} gm.

Therefore, per cell the molar concentration of glucose = $6.75 \times 10^{-14}/180 = 3.75 \times 10^{-16}$ M, and the corresponding molecular population = $3.75 \times 10^{-16} \times 6.02 \times 10^{23} = 2.26 \times 10^8$, or approximately 250,000,000 molecules of glucose per cell. Thus, calculations 1 and 2 reveal that the molecular populations of hemoglobin and glucose within the erythrocyte are practically

[4] M. F. Perutz, Ph.D., Thesis, University of Cambridge, 1940; *Nature*, 143: 73, 1939; 149: 491; 150: 324, 1942.

identical. This relationship is not at all obvious in a comparison of the values for the concentrations of hemoglobin and glucose, when they are expressed in the customary manner as respectively 34 gm and 0.075 gm per 100 ml of cells.

3. *Calculation of the Number of Hemoglobin Molecules Which Can Crowd, Closely Packed, at the Surface of an Erythrocyte*

Our spectrophotometric measurements upon whole blood[5] by means of a special cuvette of 0.007 cm depth[6] are compatible with the view that hemoglobin in the cell may be present in solution. On the other hand, various speculations are current concerning the state of hemoglobin within the red blood corpuscle. Among them is the opinion, originally expressed by Bürker,[7] that hemoglobin "is held in the corpuscle by union with the membrane."[8] This deduction arose from the interpretation of the finding that the content of the blood pigment per cell varied in different mammalian species, but was relatively constant, 2.9 to 3.4×10^{-13} gm per μ^2 unit of surface of the erythrocyte.[7] Interesting as the relation of cellular hemoglobin and corpuscular area may be, it should be clear that the use of surface area in this connection is one of personal bias. The same constancy of hemoglobin content would have been obtained if cellular hemoglobin had been calculated on the basis of cellular volume. The hypothesis of "the remarkable distribution of the coloring matter of blood on the surface of the corpuscles"[7] has found acceptance in textbooks, and has invited further speculation concerning an appropriate shape for the hemoglobin molecule—to fit it for comfortable residence at the surface of the erythrocyte—such as "long prisms, attached at one end to the membrane of cholesterol-lecithin-protein."[8] A possibly less romantic but more satisfactory shape, a rather stubby ellipsoidal platelet or slab (unaffected by the degree of hydration of the crystal) with axes $x = y = 23$ A (Ångstroms) and $z = 37.6$ A, may be deduced for the hemoglobin molecule from x-ray crystallographic data now available upon horse MHb.[4] While it may be questioned whether hemoglobin molecules within the cell need have the dimensions deduced from the crystalline material, it would appear desirable to base present inferences upon measurement rather than upon intuition. The following calculations outlaw the theory that all or a considerable proportion of cellular hemoglobin is a constituent of the membrane. Optical evidence,[9] presented in support of a variant of the above hypothesis, namely, that hemoglobin is bound to "stromatin" (stroma protein), has been refuted.[10]

(a) Surface-volume relationship of red blood cell: Owing to the peculiar biconcave disc shape of the erythrocyte, its surface area is difficult to determine with exactness, but is of the order of 140 μ^2.

The diameter, D, of a wet human erythrocyte[11] = 8.5 μ. The area (by an approximation equation[11]) = $2.4\pi(D/2)^2 = 7.55(D/2)^2 = 7.55 \times 18.07 = 136.3$ μ^2, or 1.363×10^{-10} m^2 (since 1 $\mu^2 = 10^{-12}$ m^2). (This apparently small quantity adds up to a large value for the total surface of the erythrocytes in the body. A man of 70 kilos with a blood volume of 5.6 l. has 2.8×10^{13} erythrocytes (5×10^6 per mm^3 of blood). The total surface area of the red blood cells is, therefore, $1.363 \times 10^{-10} \times 2.8 \times 10^{13} = 3.82 \times 10^3$ m^2, or 3,820 square meters, a value 2,000 times greater than the surface area of the body, which may be taken as 1.87 m^2 for a man of 70 kilos and 178 cm height). It is obvious that a sphere of the same volume as the biconcave disc erythrocyte would have a smaller surface than the latter. In the presence of added lecithin the red blood cell assumes a spherical form without change in volume, which remains at 90 μ^3 (see 1 a). The surface area of this spheroidal cell may be calculated.

Volume, V, of spherical cell = $1.333\ \pi(D/2)^3 = 4.189(D/2)^3$.

Hence, $(D/2)^3 = V/4.189 = 90/4.189 = 21.47$ μ^3, and $(D/2) = 2.78$ μ.

Therefore, surface of spherical cell = $4\pi(D/2)^2 = 12.57(D/2)^2 = 12.57 \times 7.73 = 97.3$ μ^2, or surface = $3V/(D/2) = 3 \times 90/2.78 = 97.3$ μ^2. This area is smaller than that of the corresponding biconcave disc cell of the same volume by a factor of 1.4 (calculated from 136.3/97.3). It may be assumed that the red blood cell gets along with its peculiar shape, and various physical as well as physiological advantages have been attributed[11] to the biconcave disc form. Whatever the merits of this shape may be, it is clear that it provides a larger surface per volume. Thus, per hemoglobin molecule or per any other molecule within the erythrocyte a larger cell surface is available in the biconcave disc in comparison with the spheroid of the same volume. This fact, however, should not be assumed to favor the concept of membrane-bound hemoglobin, though it may have a bearing upon the diffusion of substances, such as oxygen, from the periphery into the interior of the cell.

[5] D. L. Drabkin and R. B. Singer, *Jour. Biol. Chem.*, 129: 739, 1939.

[6] D. L. Drabkin and J. H. Austin, *Jour. Biol. Chem.*, 112: 105, 1935–36.

[7] K. Bürker, *Sitzb. preuss. Akad. Wiss.*, 140, 1922; *Naturwissenschaften*, 11: 512, 1923.

[8] A. P. Mathews, "Physiological Chemistry. A Text-Book for Students," 6th edition, Baltimore, pp. 744–745.

[9] G. A. Adams, *Biochem. Jour.*, 32: 646, 1938.

[10] D. Keilin and E. F. Hartree, *Nature*, 148: 75, 1941.

[11] E. Ponder, in O. Glasser, "Medical Physics," Chicago, 1944, p. 1203.

(*b*) The number of molecules of hemoglobin which can be packed next to each other at the surface of a red blood cell:

Surface area of erythrocyte = 136.3 $\mu^2 = 1.363 \times 10^{10}$ A^2 (since 1 $\mu^2 = 10^8$ A^2).

The dimensions of the hemoglobin molecule, based on x-ray crystallographic measurements of the unit cell of horse MHb, containing 2 molecules (Perutz[4]), are: length (*b*-axis) = 64 A, width (in direction of *a*-axis) = approximately 48 A, and thickness (perpendicular to *c* plane) = 36 A. Assuming that the smallest surface of the molecule is oriented towards or attached to the corpuscle membrane, and treating it as a quadrilateral, an approximation of this area = $48 \times 36 = 1.728 \times 10^3$ A^2. Therefore, the largest number of hemoglobin molecules which conceivably could be accommodated at the surface of the erythrocyte = $1.363 \times 10^{10}/1.728 \times 10^3 = 7.89 \times 10^6$, or approximately 8,000,000 molecules. This is less than 3 per cent. of the total number of hemoglobin molecules in the red corpuscle ($7.89 \times 10^6 \times 100/2.76 \times 10^8 = 2.86$ per cent.), and the calculation no doubt overestimates the number of molecules of hemoglobin which can crowd together in a given space. The hemoglobin molecules would be packed tighter than sardines—with no room for oil. No account has been taken of such factors as the water of hydration of hemoglobin, or the influence of the presence of other molecular species within the erythrocyte which probably would operate against the possibility that a single species of molecules could collect side by side. It may be pointed out that although the hemoglobin molecule is a relatively rigid structure,[4] the red corpuscle is not. If the biconcave disc should assume a spheroidal form (a reversible process), there would be a contraction in surface to 9.73×10^9 A^2 (see above, 3 *a*). This must lead to a tighter packing of materials fixed at the surface—an impossibility if the rigid structures are already tightly packed side by side—or there must be a displacement of substances from the surface to the interior of the erythrocyte. It is therefore probable that only 2 per cent. or less of the total hemoglobin ($9.73 \times 10^9/1.728 \times 10^3 = 5.62 \times 10^6$, out of a total of 2.76×10^8 molecules) can be anchored at the surface of the red cell, and the non-rigidity of the corpuscular structure appears to be the best argument against membrane-bound hemoglobin.

(*c*) The volume occupied by corpuscular hemoglobin:

The percentage of cellular volume occupied by hemoglobin may be calculated from the grams of hemoglobin per 100 ml of corpuscles divided by the density of hemoglobin in solution[12] = 34/1.344 = 25.3 per cent. This value is consistent with that of 70.7 per cent. for corpuscular water (from 71 gm of water per 100 ml of erythrocytes[13] times the density of water at 38° C = 0.9954), and leaves a value of 4 per cent. for the space occupied by all the other constituents of the red blood cell. An appreciably tighter squeeze than this may be inferred from calculations based on the molecular volume of hemoglobin, deduced from the x-ray dimensions,[4] $= 64 \times 48 \times 36 = 1.11 \times 10^5$ A^3, which gives a value which is 34 per cent. of the volume of the corpuscle ($1.11 \times 10^5 \times 2.76 \times 10^8 \times 100/9 \times 10^{13} = 34$ per cent.). In calculating the above volume, the molecular shape was assumed to be that of a quadrilateral slab. Since the form of the hemoglobin molecule is rather that of an ellipsoid,[4] the volume is probably smaller, and, making an allowance of 15 per cent., $= 9.4 \times 10^4$ A^3. This yields a value = 28.8 per cent. of the corpuscular volume, and affords only 0.5 per cent. of the total space for all the other erythrocyte populations besides hemoglobin and water. Hence, with inferences based on deductions from x-ray data, whether the value of 34 or 29 per cent. is chosen for the percentage of total space needed for the accommodation of hemoglobin, it appears that some water molecules must occupy the same space as hemoglobin molecules, *i.e.*, that the hemoglobin molecule is "hydrated" within the corpuscle. The above x-ray data are upon air-dried hemoglobin, which probably contains some residual water. Its specific gravity is 1.27 rather than 1.344. The lower density has been explained[12] as mainly owing to the hydration of the crystal, and the best recent measurements suggest[14] that protein crystals may contain appreciable water of hydration, from 0.5 to 0.8 gm per gm of protein. Such values applied to hemoglobin would approximately double the molecular volume. However, the hydration of the crystal affords little insight into the state of hydration of the protein in solution. Only negligible information is available as to the intimacy of association of water and a protein such as hemoglobin in solution. If the water of hydration of a protein in solution be defined as "bound" in the sense that the molecules of water associated with the protein no longer exercise their normal solvent properties, it must be concluded from accurate vapor pressure measurements[15] on centrifuged corpuscles that such water of hydration is of negligible magnitude and that the total water of the erythrocyte is practically all "free." On the other hand, it is recognized[14] that

[12] G. S. Adair and M. E. Adair, *Proc. Roy. Soc. Lond.*, B 120: 422, 1936.

[13] P. M. Hald and A. J. Eisenman, *Jour. Biol. Chem.*, 118: 275, 1937.

[14] D. Crowfoot, *Chem. Rev.*, 28: 215, 1941; T. L. McMeekin and R. C. Warner, *Jour. Am. Chem. Soc.*, 64: 2393, 1942.

[15] A. V. Hill, *Proc. Roy. Soc. London.*, B 106: 477, 1930.

not only water but salts can permeate the protein crystal structure, and it has been postulated[16] that if not water *per se* but water and solutes become associated with protein in solution, measurements involving the colligative properties would not disclose the true situation. It may well be that the ability of water plus solutes to permeate into spaces within the protein molecule may endow a special functional significance upon proteins in the cellular architecture. At any rate, there can be no doubt that even a simple cell such as the mammalian erythrocyte has a closely packed molecular population, affording intimate contact of molecules of different species. The question whether corpuscular hemoglobin is in solution or in some metastable state approaching incipient crystallization, as it appears to be in the erythrocyte of the white rat, can not be decided at present.

4. *Calculation of Molecular Populations of Dissolved and of "Bound" Oxygen, O_2, in an Erythrocyte*

(*a*) Dissolved O_2: From the absorption or solubility coefficient, α_{O_2}, at 38° C in plasma = 0.024 (*i.e.*, 0.024 ml of gas dissolved in 1 ml, at N.T.P.), and the partial pressure of O_2 in arterial blood = 97 mm of Hg, the amount of oxygen in solution in blood plasma is reckoned: $0.024 \times 100 \times 97/760 = 0.306$ ml per 100 ml of plasma, or 3.06 ml per 1,000 ml or per 10^{15} μ^3.

Assuming equilibrium in respect to dissolved O_2 between the water of plasma and water of the corpuscles, the volume of corpuscular dissolved O_2 is taken as proportional to the respective percentages of water, 93.7 per cent. for plasma and 71 per cent. for cells.[13] Hence, oxygen in solution in the corpuscles $= 3.06 \times 71/93.7 = 2.32$ ml per 10^{15} μ^3, and dissolved O_2 per corpuscle of 90 μ^3 volume $= 2.32 \times 90/10^{15} = 2.09 \times 10^{-13}$ ml, at 38° C. (This value may be an overestimation. It implies that the water of the red cells is as free to dissolve O_2 as the water of plasma. Direct determinations of dissolved oxygen in erythrocytes, presumably owing to difficulties in such analyses, are unavailable. An inert gas, hydrogen, is 93 per cent. as soluble in corpuscles as in serum[17].)

Since 1 gram mole of gas occupies 22,400 ml, at N.T.P., the molar concentration of dissolved $O_2 = 2.09 \times 10^{-13}/2.24 \times 10^4 = 9.33 \times 10^{-18}$ M per red blood cell.

Therefore, the molecular population in dissolved O_2 of a single erythrocyte $= 6.02 \times 10^{23} \times 9.33 \times 10^{-18} = 5.62 \times 10^6$, or approximately only 5,500,000 molecules of dissolved oxygen per arterial corpuscle.

(*b*) Molecules of O_2 "bound" to hemoglobin, and ratio of bound to free O_2 in arterial cells: The hemoglobin molecule contains 4 atoms of Fe, and, at full saturation (complete conversion to oxyhemoglobin), there will be 4 molecules of O_2 bound per 4 atoms of Fe in 1 molecule of hemoglobin. With the values of 98 per cent. for the saturation of arterial hemoglobin[1] and 2.76×10^8 for the number of hemoglobin molecules per cell (see 1 *b*), the bound $O_2 = 2.76 \times 10^8 \times 4 \times 0.98 = 1.08 \times 10^9$, or approximately 1,000,000,000 molecules of bound oxygen per corpuscle in arterial blood. Therefore, in an arterial erythrocyte the ratio of bound O_2 to free $O_2 = 1.08 \times 10^9/5.62 \times 10^6 = 192/1$. In other words, in a red corpuscle of arterial blood there is 192 times more oxygen united with hemoglobin than there is free in solution (physically dissolved). In venous blood, at 38° C, with 80 per cent. saturation of hemoglobin and an oxygen tension of 40 mm of Hg, one may calculate (in the same way as above) that there are, per corpuscle, 8.84×10^8 molecules of bound to only 2.31×10^6 molecules of free O_2, yielding a ratio of bound O_2 to free O_2 of 383/1. Hence, in a red cell of venous blood there is 383 times more oxygen combined with hemoglobin than there is free in solution. Such values are more pertinent and revealing with reference to the nature of the hemoglobin-oxygen equilibrium than values for bound and free oxygen in terms of volumes per cent. in whole blood, particularly when the value for free O_2 refers to plasma alone. Representative values in terms of volumes per cent. are: 20.5 for oxygen capacity (related to complete saturation of 15.3 gm of hemoglobin per 100 ml of whole blood) and 0.306 for free oxygen in 100 ml of plasma. The ratio of $20.5/0.306 = 67/1$ does not reveal, without the application of suitable corrections, the situation in respect to bound and free oxygen in the erythrocyte.

The number of molecules of dissolved O_2 in an erythrocyte, as disclosed by the calculations, is surprisingly small. Under normal conditions, in arterial blood corpuscles there is just enough oxygen to nearly saturate the hemoglobin, but not quite. With a minimum utilization rate of about 250 ml of O_2 per minute for an adult man at rest, and up to 2,500 ml per minute in strenuous exercise, and with at most only 15.4 ml of total dissolved O_2 in 5.6 liters of blood (70 kilo man, with 3.08 liters of plasma and 2.52 liters of cells), it is obvious that in providing oxygen a cooperative effort must exist among the carrier (hemoglobin), the pumping system (heart and circulation) and the source (respiration). So far as the carrier is concerned two factors are of dominant importance—(1) provision of sufficient dissolved oxygen in time, and (2) maintenance of an active form of hemoglobin, capable of uniting reversibly with oxygen. Physiologists do not appear to

[16] B. S. Neuhausen, *Jour. Biol. Chem.*, 51: 435, 1922.
[17] D. D. Van Slyke and J. Sendroy, Jr., *Jour. Biol. Chem.*, 78: 801, 1928.

have stressed the fact that functionally the erythrocyte is peculiarly adapted to aid hemoglobin in both of the above connections. The red blood corpuscle has a very low, almost negligible oxidative metabolism (consumes very little oxygen) in comparison with other cells. Indeed, it has been described, unfortunately, as "dead." If the erythrocyte had an active oxidative metabolism it would itself consume the dissolved oxygen (of which there is none to spare), and hemoglobin would be useless as a carrier. To illustrate what could occur if cells which consumed oxygen actively were present in blood, the following simple and instructive experiment may be performed: Mix equal parts of a solution (1 volume of cells in a total of 10 volumes), prepared from washed erythrocytes (100 per cent. of oxyhemoglobin), with a 10 per cent. suspension of washed, tapioca-free yeast. Dilute 1 volume of the mixture to a total of 2.5 volumes with distilled water, and allow to stand in an open test-tube at room temperature for 20 minutes. The color of the mixture changes to deep purple, owing to the formation of deoxygenated (reduced) hemoglobin. The deoxygenation of the hemoglobin with an active yeast suspension will be practically complete. (The yeast may be centrifuged off, and the hemoglobin-oxyhemoglobin mixture determined spectrophotometrically.) If the tube is shaken with air, the upper layer turns red (reoxygenation of the hemoglobin). On further standing reversion to reduced hemoglobin again occurs. This reversible change may be induced many times. To show that the phenomenon is that of oxygen consumption by the yeast cells rather than reduction by some active reducing agent in the yeast, a control experiment may be carried out, using a 1:10 solution of MHb, prepared by adding an equivalent of ferricyanide to the blood sample. The yeast now has no effect upon the color of the solution. MHb is not reduced by any substance in the yeast. If the yeast is separated from the MHb solution by centrifuging and added to a solution of oxyhemoglobin, the process of deoxygenation may again be demonstrated. Similar results can be obtained by substituting a fresh homogenate of liver for the yeast cells. It is, therefore, fortunate that mammalian red blood corpuscles do not have an active oxidative metabolism. Paradoxically, however, it is also fortunate that erythrocytes are not metabolically inert. They are provided with all the necessary biocatalysts—coenzymes I and II, the oxidizing enzyme, enolase, etc.—to support an active glycolytic metabolism. And, it is probable that glycolysis within the erythrocyte is an essential functional property, necessary for maintaining a high concentration of active hemoglobin *in vivo*. As mentioned in the opening paragraphs, glycolysis and disappearance of MHb (oxidized, inactive hemoglobin) go hand in hand. Iodoacetate and fluoride, known to poison certain of the above enzymes, inhibit both the breakdown of glucose and the reduction of MHb to active hemoglobin, capable of uniting with oxygen. In the conservation of dissolved oxygen the red corpuscle is merely a passive agent, in its capacity for shifting or keeping the equilibrium, oxyhemoglobin $\rightleftharpoons$ hemoglobin $\rightleftharpoons$ methemoglobin, far to the left it has an active functional rôle, which deserves recognition in hemoglobin physiology.

5. *Calculation of Molecular Population of Water in an Erythrocyte*

There are 710 gm of water per 1,000 ml of blood corpuscles,[13] or per 10^{15} μ^3, and the mol. wt. of water = 18.02.

Hence, neglecting the density at 38° C = 0.993, the molar concentration of water = 710/18.02 = 39.4 M per 10^{15} μ^3.

Therefore, the molecular population in water of a single corpuscle of 90 μ^3 volume = $39.4 \times 6.02 \times 10^{23} \times 90/10^{15} = 2.14 \times 10^{12}$, or approximately 2,000,000,000,000 molecules of water per red blood cell. The census indicates that water is by far the largest segment of the molecular population of cells. In the erythrocyte, which contains less water than many other types of cells, there is approximately an 8,000 times larger number of water molecules than molecules of hemoglobin ($2.14 \times 10^{12}/2.76 \times 10^8 = 7.75 \times 10^3$). An appreciably greater difference than this exists in the molecular populations of water and of dissolved oxygen, the ratio of the two components being 400,000 to 1, ($2.14 \times 10^{12}/5.62 \times 10^6 = 3.81 \times 10^5/1$). In other words, the number of molecules of free oxygen is less than 0.0003 per cent. of the number of molecules of water. Since water may not be an entirely silent partner in various metabolic transactions, relationships such as the above invite speculation.

6. *Relationship of Insulin and Glucose: the Relative Frequency of Two Simultaneous Events*

Thus far, the calculations have been limited to the molecular populations of the erythrocyte. Here, an example is furnished of the insight afforded into a more general biological relationship, that of insulin and glucose, when expressed in molecular terms. In a depancreatized dog a normal level of blood sugar can be maintained by a simultaneous, constant injection intravenously of *0.07 unit* of insulin and *0.25 gm* of glucose per kilo of body weight per hour,[18] the latter value representing the normal utilization rate

[18] S. Soskin and M. D. Allweiss, *Am. Jour. Physiol.*, 110: 4, 1934–35.

of sugar. An approximately similar relationship of insulin and glucose is obtained from the hormone and sugar requirements of a severely diabetic child. The values of *0.07 unit* and *0.25 gm* are thus bracketed together, and have some usefulness therapeutically, but they have little obvious further significance. However, this empirically established relationship of insulin and glucose supplies fundamental data, needed in the following calculations:

(*a*) Molar equivalent of 0.07 unit of insulin.:

1 mg of pure, crystalline insulin = 22 units, and mol. wt. of insulin = 35,100.

Hence, 1 unit = 1/22 = 0.0454 mg, and 0.07 unit = $0.0454 \times 0.07 = 0.00318$ mg, or 3.18×10^{-6} gm.

Therefore, the molar equivalent of 0.07 unit of insulin = $3.18 \times 10^{-6}/3.51 \times 10^{4} = 9.06 \times 10^{-11}$ M.

(*b*) The molar equivalent of 0.25 gm of glucose (mol. wt. = 180) = $0.25/180 = 1.39 \times 10^{-3}$ M.

Therefore, the molecular ratio of glucose to insulin = $1.39 \times 10^{-3}/9.06 \times 10^{-11} = 1.53 \times 10^{7}/1$. Thus, per molecule of insulin required by the completely diabetic (or produced by the normal organism), 15,300,000 molecules of glucose are utilized (fully oxidized) per hour, or 255,000 molecules per minute. Attention may be drawn to the fact that care has been taken to state the relationship of insulin and glucose in such a way as not to imply that insulin is necessarily directly concerned with the oxidation of glucose. The exact functions and mode of action of insulin are still open questions. The calculations are of interest and valid as an indication of the relative frequency or magnitudes of two simultaneous processes, the production of insulin and the oxidation of glucose, for which some interrelationship is probable in the organism. If insulin is concerned with glucose oxidation, the value of 255,000 molecules of glucose taken care of by 1 molecule of insulin per minute assumes the character of a turnover number. As such, it is unusually high, and has the added distinction of being referable directly to the living organism. Insulin does a good job, the magnitude of which is defined clearly by the molecular relationship of the hormone and sugar.

Summary

Calculations have been presented to illustrate the insight which may be gained from the development of concepts of biological magnitudes upon the basis of molecular dimensions.

OBITUARY

RECENT DEATHS

ROBERT ELMER HORTON, consulting hydraulic engineer to the Tennessee Valley Authority and chairman of the Board of Consultants on Flood Control of the U. S. Department of Agriculture, died in his seventieth year on April 22.

DR. MARTIN H. ITTNER, chief chemist of the Colgate-Palmolive-Peet Company, died on April 22. He was seventy-four years old.

ARTHUR ROBERT HINKS, astronomer and since 1915 secretary of the Royal Geographical Society, died on April 18 at the age of seventy-one years.

DR. HANS SACHS, formerly professor of immunology at the Medical School of the University of Heidelberg, who was connected with Trinity College in Dublin, where he had a fellowship, died on March 28.

SIR AMBROSE FLEMING, known for his work in wireless, radio and telegraph developments, died on April 19 at the age of ninety-five years.

SCIENTIFIC EVENTS

NEW MECHANICAL ENGINEERING BUILDING AT THE CALIFORNIA INSTITUTE OF TECHNOLOGY

THERE has just been completed for the use of the Mechanical Engineering Department of the California Institute of Technology a five-story, reinforced concrete building. This follows the usual type of construction with three floors above the ground level and two floors below. However, the so-called first floor is five feet above the ground level so that by the use of light wells, daylight is supplied to the entire first basement, and because of a portion of the first basement floor being omitted, sunlight actually reaches the very lowest floor level. The building contains two very good drafting rooms on the top floor with excellent daylight lighting and also is illuminated by fluorescent lights. These lights are arranged on the ceiling in diagonals so that with the drafting tables located square with the room no shadows will be cast. There are two very good classrooms and a lecture room equipped with a projection lantern and screen and a demonstration table supplied with water, gas, compressed air and 110- and 220-volt AC current. The building also contains the offices for the members of the instructing staff and a good portion of the equipment of the Laboratory of Mechanical Engineer-