THE DISTRIBUTION OF THE CHROMOPROTEINS, HEMOGLOBIN, MYOGLOBIN, AND CYTOCHROME c, IN THE TISSUES OF DIFFERENT SPECIES, AND THE RELATIONSHIP OF THE TOTAL CONTENT OF EACH CHROMOPROTEIN TO BODY MASS*

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It is recognized that the over-all energy metabolism and, hence, the oxygen consumption bear a relationship of proportionality, not to body mass, but to a fractional exponent of this quantity. The skin surface area has a similar mathematical relation to the body mass. Thus, approximately equal values for caloric output (heat loss) or for oxygen intake per unit time are obtainable in species of different size, when these measurements are related either to a fractional exponent of their body masses (2, 3) or to their measured or calculated surface areas (4, 5). One avoids the direction of thought into unproductive channels by not pursuing the academic arguments (2, 3) as to which of these bases of reference (fractional exponent of body mass or surface area) is the more correct or valid in expressing the basal metabolism. On the whole, similar deductions can be arrived at from each empirical reference base, and each suffers from similar disadvantages and defects, which are largely owing to the fact that they appear to support a concept, very probably incorrect, that all kinds of protoplasm are the same. Nevertheless, valuable clinical inferences have been made in man by the use of the surface area base, and, without undue faith in their ultimate significance, both surface area and fractional exponent of the body mass are of service in the interpretation of the over-all metabolism of different species.

With reference to specific metabolites or some of the agencies of metabolism and their relationship to body mass, the present knowledge is fragmentary. Dreyer et al. (6, 7), who first introduced the fractional expo-

* Most of this work was carried out under contract between the Office of Naval Research and the University of Pennsylvania. Preliminary reports have been made at the meetings of the Federation of American Societies for Experimental Biology at Atlantic City, March 15-19, 1948 (1), and at the Sir Joseph Barcroft Memorial Conference on Haemoglobin, Cambridge, England, June, 1948 (1).
nent of the body mass, $W^{0.70}$ to $W^{0.71}$, as a point of reference, deduced that the blood volumes (and, hence, the total hemoglobin content) of different species were the same when expressed on this basis. This deduction, as will be seen, appears untenable. In an early study by the writer (8), it was found that the output of the normal urinary pigment, urochrome (whose chemistry remains unknown), in several different sized species was directly proportional to their surface areas. The minimal nitrogen excretion (formerly designated "endogenous nitrogen"), attained on a high calorie-no protein regimen, has been shown to be constant per kilocalorie of heat loss in different species, and, therefore, proportional to an exponential function of the body weight, as $W^{0.7}$ (9, 10). The neutral sulfur excretion (a probable component of which is urochrome) in the fasting state has a similar relationship (cf. (2)). More recently, in analyses limited to epithelial tissues, Rosenthal and Drabkin (11) reported the suggestive observation that the concentration of cytochrome c (as in kidney cortex) had an inverse relationship to the body size of the species.

In view of the recognized importance of hemoglobin, myoglobin, and cytochrome c (all conjugated complexes of the common iron protoporphyrin type III) in oxygen homeostasis, it has appeared desirable to secure more exact and complete information upon the total amounts of these chromoproteins and their distribution in various tissues of different mammalian species. Such quantitative data could be expected to disclose the possible existence and the character of any relationships between each pigment and such factors as body mass. Aside from this, such information is valuable in developing valid concepts of the magnitudes of the metabolic transactions involved (1, 12), as well as in shedding further light on the discrete metabolic functions of the individual chromoproteins. Complete data upon the total hemoglobin, myoglobin, and cytochrome c, and the distribution of the latter, in the body of the rat have already been reported from this laboratory (13). In the present paper, selected comparative data on different species, gathered over a number of years, will be presented, and their interpretation discussed.

Methods

Analytical Procedures—The concentration of hemoglobin was determined spectrophotometrically as cyanmethemoglobin, with our usual constant $\varepsilon$ ($c = 1 \text{ mm per liter}, d = 1 \text{ cm.}) = 11.5$ at wave-length 540 m$\mu$ (14). Since hemoglobin is restricted to the erythrocytes, the total hemo-

\footnote{In the notation $\varepsilon$ ($c = 1 \text{ mm per liter}, d = 1 \text{ cm.}) = (1/(c \times d)) \times \log I_0/I$, where the concentration, $c$, is expressed in mm per liter, the depth, $d$, in cm., the intensity of incident radiation, $I_0$, is 1.0, and the intensity of the transmitted flux, $I$, is expressed as a fraction of unity. The various spectrophotometric constants used
globin may be obtained from the concentration of this chromoprotein per unit volume of blood times the total blood volume. In the rat (13), dog, and man, fairly reliable values for the latter are calculated from the plasma volume (measured by the dye, T1824, dilution method (16)) and the fraction of plasma, determined by hematocrit. For the cow and horse the evaluations of blood volume are less reliable, but are regarded as sufficiently close for present purposes. The values for blood volume, which have been used, are furnished in Column 1, Table IV.

A quantitative volumetric application (13) of Morgan's modification of Theorell's method (17) was adapted for the extraction of myoglobin. For quantitative estimation of this pigment, the isolation procedure needs to be carried only to the stage of separation of contaminating hemoglobin, with solution of the myoglobin in 3 M phosphate buffer of pH 6.6 (13). The chromoprotein was converted to cyanometmyoglobin by addition of ferricyanide and cyanide, and its concentration determined spectrophotometrically, with the constant \( \epsilon (c = 1 \text{ mm per liter}, d = 1 \text{ cm.}) = 11.3 \) at wave-length 540 m\( \mu \) (13, 15). As there is no indication at present that organs such as liver, spleen, and kidney may contain tissue hemoglobin analogues, myoglobin was assumed to be limited to skeletal, cardiac, and smooth muscle. The chromoprotein content of the latter was not determined, since it was regarded as negligible in comparison with the total myoglobin of the skeletal muscle. The total content of myoglobin was obtained from its concentration per unit mass of muscle times the total muscle mass. The values used for the latter are approximations. They varied in the different species between 36 and 45 per cent of the body mass and are given in Column 1, Table IV.

Cytochrome c in individual tissues was determined by the direct microspectrophotometric capillary cuvette-diaphragm technique of Rosenthal

by us for the hemin pigments have now been established upon, and are referable to, an iron basis (15). The spectrophotometric determination of cyanmethemoglobin and cyanometmyoglobin is the most direct and unequivocal determination of hemin iron. The molecular magnitudes of reference are the 1 iron atom equivalent weights: 16,709 for hemoglobin (0.335 per cent Fe), 16,400 for myoglobin (0.340 per cent Fe), and 13,000 for cytochrome c (0.43 per cent Fe).

In the original step of the procedure, small pieces of muscle, freed of ligaments and obvious fat, are ground finely and extracted in one-half their weight of water in a Waring blender. Extraction is continued overnight at refrigerator temperature, and the crude aqueous extract recovered by pressure exerted on the material placed in a muslin sac. In the case of cardiac muscle it has been demonstrated that, although myoglobin readily passed into the water, cytochrome c was totally retained in the press cake, from which it could be isolated, but only by the use of special extracting agents, dilute trichloroacetic (18) or sulfuric acid (19). This simple observation suggests that myoglobin may be relatively "free," whereas cytochrome c may be "bound" in the cellular structure.
and Drabkin (19). The organ content of this chromoprotein was obtained from the concentration per gm. of wet weight of tissue times the weight of the organ in gm. The summation of the values in the separate tissues yields the total content of cytochrome c in the body. This has been done for the rat (13) and for man (Table II). In these two species, the body content of cytochrome c has been found to be predicted with sufficient reliability from the content of the pigment in the skeletal muscle mass divided by the factor 0.8, or from its content in the combined muscle, heart, liver, and kidney mass, divided by the factor 0.93. In the dog, each of these methods of calculating the content of cytochrome c has been shown to yield the same results. In the larger species, cow and horse, it was assumed that a similar relationship of the content of this pigment in skeletal muscle to that of the whole body was valid, and the factor 0.8 was employed to approximate the total cytochrome c from its quantity in the muscle mass. The method of Rosenthal and Drabkin (19) and the original Keilin and Hartree procedure (18) have been found unreliable for the determination of the cytochrome c concentration (presumably very small) in human blood. Adsorption on other proteins or blood constituents may be the source of the difficulty, at present unresolved, but the recovery of cytochrome c added to the blood was poor. The cytochrome c content of human blood (Table II) is, therefore, uncertain.

Specimens of human tissues were largely obtained as promptly as possible after death from causes believed not to affect adversely the composition of the tissues subjected to analysis. The tissues of the cow and horse were obtained at the sacrifice of these animals, necessitated by injury or intercurrent disease. The body surface areas of the individuals (Column 1, Table IV) were calculated with the following formulas: rat, Rubner (20); dog, Cowgill and Drabkin (5); man, Dubois and Dubois (4); cow, Brody and Elting (21); and horse, Seuffert and Hertel (22).

Results

Table I contains comparative values, which illustrate the close parallelism of data on the rate of oxygen consumption (which reflects oxidative activity) and on cytochrome c concentration in tissues of the rat. Although exact comparison is hampered by the fact that oxygen consumption is referred to the usual "terminal" dry weight (in the Warburg analytical procedure), whereas the concentration of cytochrome c is on the basis of the initial dry weight, nevertheless, the relationship of the two is striking. Each is low in tissues with low oxidative ability (red blood corpuscles and skin), each has intermediate values in tissues with moderate or fairly high metabolism (skeletal muscle, brain, and liver), and each has high values in tissues which maintain a continuous high level of work,
requiring continuous large energy expenditure (kidneys and heart). Hence, with reference to their oxidative ability, the protoplasms of these various tissues are remarkably different. The range between the most and least active is some 200- to 300-fold: \( Q_{O_2} \) (heart)/\( Q_{O_2} \) (erythrocytes) = 200 (from 20/0.1), and cytochrome c (heart)/cytochrome c (erythrocytes) = 242 (from 1.940/0.008).

The relationship of oxygen consumption and cytochrome c concentration is, doubtless, the basis for our earlier finding (23) that the determination of cytochrome c can be used as an index of the activity of cytochrome oxidase in tissues. The proportionality of oxygen consump-

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportionality of Cytochrome c Concentration and Oxygen Consumption in Rat Tissues</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( Q_{O_2} )</th>
<th>Cytochrome c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per mg. dry weight</td>
<td>Per gm. wet weight</td>
</tr>
<tr>
<td></td>
<td>( \gamma )</td>
<td>( \gamma )</td>
</tr>
<tr>
<td>Red blood corpuscles</td>
<td>0.1</td>
<td>0.008</td>
</tr>
<tr>
<td>Skin</td>
<td>1-2</td>
<td>0.051</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>6</td>
<td>0.381</td>
</tr>
<tr>
<td>Brain cortex</td>
<td>10</td>
<td>0.375†</td>
</tr>
<tr>
<td>Liver</td>
<td>10†</td>
<td>0.60/2‡</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td>20</td>
<td>1.433§</td>
</tr>
<tr>
<td>Heart</td>
<td>20‡</td>
<td>1.940§</td>
</tr>
<tr>
<td>Retina</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

*Microliters of \( O_2 \) consumed per mg. of dry weight of tissue per hour; approximate magnitudes related to "terminal" dry weight, i.e. dry weight after the analytical run of the tissue slice in the Warburg apparatus.

† For direct comparison with \( Q_{O_2} \) values.
‡ From data of Rosenthal and Drabkin (11).
§ From data of Crandall and Drabkin (13).

In Table II are recorded our mean values for the cytochrome c concen-
tration and content of different human organs, calculated for a reference body mass of 70 kilos. The summation of these values yields a value of only 0.8 gm. for the total body content of this chromoprotein. The analytical results upon individual tissues are, with some exceptions, in es-

**Table II**

**Total Cytochrome c Content of Adult Human Male**

The values are calculated for a reference body weight of 70 kilos from the means of analytical data upon the individual tissues of three to eight subjects.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Weight of organ*</th>
<th>Per cent of body weight</th>
<th>Per gm. tissue, wet weight</th>
<th>Total in organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscles</td>
<td>29,400 gm.</td>
<td>42.0</td>
<td>22</td>
<td>646.8</td>
</tr>
<tr>
<td>Heart</td>
<td>330</td>
<td>0.47</td>
<td>136</td>
<td>44.9</td>
</tr>
<tr>
<td>Skin†</td>
<td>12,600 gm.</td>
<td>18.0</td>
<td>2.1</td>
<td>26.5</td>
</tr>
<tr>
<td>Liver</td>
<td>1,610</td>
<td>2.3</td>
<td>15</td>
<td>24.2</td>
</tr>
<tr>
<td>Brain</td>
<td>1,400</td>
<td>2.0</td>
<td>14</td>
<td>19.6</td>
</tr>
<tr>
<td>Abdominal organs†</td>
<td>1,400</td>
<td>2.0</td>
<td>0.5§</td>
<td>9.1</td>
</tr>
<tr>
<td>Blood</td>
<td>5,910†</td>
<td>8.45</td>
<td>0.9¶</td>
<td>5.3</td>
</tr>
<tr>
<td>Kidneys</td>
<td>330</td>
<td>0.47</td>
<td>12</td>
<td>4.0</td>
</tr>
<tr>
<td>Lungs</td>
<td>490</td>
<td>0.70</td>
<td>1.6§</td>
<td>0.8</td>
</tr>
<tr>
<td>Skeleton</td>
<td>10,500</td>
<td>15.0</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>6,030††</td>
<td>8.61</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>70,000 gm.</td>
<td>100</td>
<td>781</td>
<td></td>
</tr>
</tbody>
</table>

* Except for the blood and kidneys, organ weights are based on values reported by Skelton (26), adjusted to a total body mass of 70 kilos.
† "Whole" skin, including subcutaneous tissue.
‡ Exclusive of liver and kidneys.
§ From data of Rosenthal and Drabkin (11).
¶ Value based on plasma volume of 3150 ml., numerically equal to 4.5 per cent of the body weight, 0.56 for fraction of plasma (from hematocrit), and a specific gravity for whole blood of 1.055. Skelton’s value (26) for blood mass is 40 per cent too low.
¶¶ This value is questionable (see “Methods”).
** Cytochrome c concentration not determined; assumed to be negligible in these tissues.
†† The difference between the total body mass of 70,000 gm. and the summation of the other components.

sentinal agreement with corresponding data reported by Greenstein et al. (27). Minor discrepancies include the heart, in which our results are significantly higher, and the kidneys and lungs, in which they are somewhat lower. The concentration of this pigment in all human tissues is
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appreciably lower than that in corresponding organs of the rat, reported by Crandall and Drabkin (13). However, a comparison of the data on the rat (last column, Table I) and man (fourth column, Table II) revealed that the differences in concentration of cytochrome c were very much greater in the case of some tissues than of others. Thus, rat liver and kidney had respectively 15- and 30-fold greater concentrations of the chromoprotein than the corresponding organs in man. On the other hand, the concentrations of cytochrome c were only 3 and 5 times greater in rat heart and skeletal muscle than in the same human tissues. Of main present pertinence was the total body content of cytochrome c. A 70 kilo man contained 781 mg. of the pigment (Table II), as compared with 14.4 mg. found earlier (13) in a 0.25 kilo rat. There was an obvious disproportionality between the body mass and cytochrome c ratios: mass (man)/mass (rat) = 70/0.25 = 280; cytochrome c (man)/cytochrome c (rat) = 781/14.4 = 54.

In Tables III and IV are presented summaries of some of our data from which deductions, regarded as significant, have been drawn. The data collected in Table III afford a comparison of the concentrations of myoglobin and cytochrome c in cardiac and skeletal muscle. From the information supplied on the organ weights, the content of these chromoproteins may also be readily calculated. This comparison of myoglobin and cytochrome c is of interest from several standpoints. In muscle tissue the two pigments, one functioning in the transport (28), the other in the utilization phase of oxygen homeostasis, exist side by side, and, hence, a direct comparison is afforded of their relative intracellular concentrations in the same tissues. Furthermore, heart muscle is an organ working continuously, with a relatively high rate of metabolic activity, whereas skeletal muscle, quite quiescent in the basal state (see Table V), is capable of periodic bursts of activity, involving at times enormous energy expenditures. It has already been stated that the concentrations of cytochrome c are not as far apart in the heart tissue of different sized species as they are in some of the other tissues. It may now be seen (Table III) that the concentration of cytochrome c concerned with cellular oxidative activity (or capacity) is 3 to 6 times higher in the organ of continuous work, the heart, than in skeletal muscle. The consistency of the interspecies data gives greater weight to this finding and the generalizations which may be drawn therefrom. The high concentration of cytochrome c in heart muscle is consonant with this organ's high rate of oxygen consumption (Table I), and also with recent demonstrations, by the technique of coronary sinus catheterization (29, 30), of the unusually high degree of deoxygenation of blood supplying the heart. It may also be noted that the concentration of this chromoprotein in muscle
tissue, with the exception of the horse, decreases with increasing size of the species.

The concentration of myoglobin (Table III), on the other hand, appears to have no systematic relationship to body size. It is high in those species (dog, horse) which either run fast or work hard, and it is low in

**Table III**

*Comparative Values of Myoglobin and Cytochrome c in Heart and Skeletal Muscles of Different Species*

Skeletal muscle mass in dog, man, and horse estimated as 36, 42, and 45 per cent of their respective body weights.

<table>
<thead>
<tr>
<th>Species</th>
<th>Heart</th>
<th>Skeletal muscle</th>
<th>Ratio, concentrations in heart and skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass</td>
<td>Myoglobin</td>
<td>Cytochrome c</td>
</tr>
<tr>
<td></td>
<td>gm.</td>
<td>mg. per gm.</td>
<td>mg. per gm.</td>
</tr>
<tr>
<td>Rat†</td>
<td>0.73</td>
<td>0.91</td>
<td>0.447</td>
</tr>
<tr>
<td>Dog</td>
<td>51</td>
<td>2.1</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.7</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>1.7</td>
<td>0.228</td>
</tr>
<tr>
<td>Man</td>
<td>290</td>
<td>0.108</td>
<td>26.04</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>0.148</td>
<td>28.14</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>1.4</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>330</td>
<td>1.1</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>330</td>
<td>1.1</td>
<td>0.127</td>
</tr>
<tr>
<td></td>
<td>335</td>
<td>1.0</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>0.158</td>
<td>31.92</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>1.3</td>
<td>0.128</td>
</tr>
<tr>
<td>Horse</td>
<td>2665</td>
<td>4.0</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>4.7</td>
<td>0.212</td>
</tr>
<tr>
<td></td>
<td>2805</td>
<td>5.1</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>2810</td>
<td>4.7</td>
<td>0.182</td>
</tr>
<tr>
<td></td>
<td>2837</td>
<td>4.5</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>2920</td>
<td>4.4</td>
<td>0.187</td>
</tr>
</tbody>
</table>

* Wet weight of tissue.
† Mean values from data of Crandall and Drabkin (13), calculated for an adult rat of 250 gm.
‡ These data were used to derive values for total myoglobin and cytochrome c in the dog and horse respectively in Table IV.

rat and man. Also, in the hard workers the myoglobin concentration is considerably higher in skeletal than in cardiac muscle, whereas in rat and man the concentration of this pigment is of a similar order of magnitude in the two types of muscle. The data may lend support to the view that myoglobin functions mainly in “oxygen debt” (28). On the basis
of the relative concentrations of myoglobin, disclosed by the data on the
dog and horse, it is possible that the heart has less capacity for handling
a developing oxygen debt than does skeletal muscle.

Our values for the concentration of myoglobin in the muscle tissues of
the dog are lower than those reported in early studies by Whipple (31).
This may be owing to the fact that contaminating hemoglobin, always
present in aqueous extracts of muscle, is separated completely from the
myoglobin in the present analytical technique. However, our values for
myoglobin in man are also appreciably lower than those recently reported
for human muscle by Börck (32), who used a presumably reliable ana-
lytical procedure. The data in Table III also supply information as to
the reproducibility of concentration values for the chromoproteins in
individuals within one species. The values for cytochrome c, as already
demonstrated for the rat (11, 13, 33), proved to be relatively constant
for a particular tissue, and characteristic for each species. The concen-
tration of myoglobin (and, hence, the total content of this pigment),
though fairly uniform in the heart, was quite variable in skeletal muscle
from subject to subject (dog, horse, Tables III and IV). This variabil-
ity in myoglobin may be related to the factor of exercise conditioning
thought to affect the level of myoglobin (31, 32), and a separate investi-
gation of this aspect of the problem is contemplated.

In Table IV the most salient features of the relationship of the chromo-
proteins to the body weight in five different species are presented. At-
tention is directed to the following.

1. The content of each chromoprotein, hemoglobin, myoglobin, and
cytochrome c (Column 3, Table IV), increases with increased body size,
and it is obvious that the oxygen transport chemicals, especially hemo-
globin, are present in enormously larger amounts (in terms of gm.) than
is the representative of the oxygen utilization system. By assigning a
value of unity to the content of cytochrome c, the relative abundance of
the three chromoproteins may be calculated from the data in Column 3,
and expressed as ratios of hemoglobin-myoglobin-cytochrome c. These
ratios are 222:7:1 (rat), 467:25:1 (dog), 649:85:1 (dog), 1169:44:1
becomes evident that the relative quantities of the three chromoproteins
are quite different in the different species. Two sets of values are sup-
plied for the dog and horse to indicate the variability in total myoglobin
which, nevertheless, is unusually high in the horse. In this species, the
myoglobin content is greater than 30 per cent of the hemoglobin content
(compare 1867 gm. and 5805 gm., Column 3), whereas, in rat and man,
total myoglobin is only 3 to 4 per cent of the total hemoglobin (from values
in Column 3).

2. The evaluation of the chromoprotein content per kilo of body mass
**TABLE IV**

Relationships of Total Chromoproteins to Body Mass and Surface Area

In Column 1, \( W \) = body mass, \( S \) = surface area, \( V \) = blood volume, and \( M \) = skeletal muscle mass.

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromoprotein*</th>
<th>Total (3)</th>
<th>Per kilo (4)</th>
<th>Per sq.m. (5)</th>
<th>Per ( W^{1/8} ) (6)</th>
<th>Per ( W^{2/5} ) (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat; 45 kilos, ( S ) 0.0361 sq.m., ( V ) 19.2 ml., ( M ) 0.113 kilos</td>
<td>Hemoglobin</td>
<td>3.19</td>
<td>12.76</td>
<td>88.3</td>
<td>8.42</td>
<td>9.01</td>
</tr>
<tr>
<td></td>
<td>Myoglobin</td>
<td>0.101</td>
<td>0.404</td>
<td>2.8</td>
<td>0.267</td>
<td>0.286</td>
</tr>
<tr>
<td></td>
<td>Cytochrome ( c )†</td>
<td>0.0144</td>
<td>0.056</td>
<td>0.399</td>
<td>0.038</td>
<td>0.041</td>
</tr>
<tr>
<td>Dog; 9.88 kilos, ( S ) 0.523 sq.m., ( V ) 700 ml., ( M ) 3.56 kilos</td>
<td>Hemoglobin</td>
<td>138.3</td>
<td>14.00</td>
<td>264.4</td>
<td>27.8</td>
<td>24.8</td>
</tr>
<tr>
<td></td>
<td>Myoglobin</td>
<td>7.5</td>
<td>0.76</td>
<td>14.3</td>
<td>1.51</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>Cytochrome ( c )§</td>
<td>0.240</td>
<td>0.025</td>
<td>0.476</td>
<td>0.050</td>
<td>0.045</td>
</tr>
<tr>
<td>Dog; 6.35 kilos, ( S ) 0.330 sq.m., ( V ) 508 ml., ( M ) 2.29 kilos</td>
<td>Hemoglobin</td>
<td>88.9</td>
<td>14.00</td>
<td>269.4</td>
<td>24.2</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Myoglobin</td>
<td>11.7</td>
<td>1.84</td>
<td>35.5</td>
<td>3.18</td>
<td>2.93</td>
</tr>
<tr>
<td></td>
<td>Cytochrome ( c )§</td>
<td>0.137</td>
<td>0.022</td>
<td>0.415</td>
<td>0.037</td>
<td>0.034</td>
</tr>
<tr>
<td>Man; 70 kilos, ( S ) 1.87 sq.m., ( V ) 5000 ml., ( M ) 29.40 kilos</td>
<td>Hemoglobin</td>
<td>912.8</td>
<td></td>
<td>488.1</td>
<td>46.8</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td>Myoglobin</td>
<td>34.7</td>
<td>0.50</td>
<td>18.6</td>
<td>1.77</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>Cytochrome ( c )§</td>
<td>0.781</td>
<td>0.011</td>
<td>0.417</td>
<td>0.040</td>
<td>0.032</td>
</tr>
<tr>
<td>Heifer; 182 kilos, ( S ) 2.92 sq.m., ( V ) 14,560 ml., ( M ) 81.9 kilos</td>
<td>Hemoglobin</td>
<td>2215.0</td>
<td>12.17</td>
<td>758.6</td>
<td>57.9</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td>Myoglobin</td>
<td>307.0</td>
<td>1.69</td>
<td>105.1</td>
<td>8.03</td>
<td>6.20</td>
</tr>
<tr>
<td></td>
<td>Cytochrome ( c )§</td>
<td>1.24</td>
<td>0.0068</td>
<td>0.424</td>
<td>0.032</td>
<td>0.025</td>
</tr>
<tr>
<td>Horse; 500 kilos, ( S ) 6.62 sq.m., ( V ) 45,000 ml., ( M ) 225 kilos</td>
<td>Hemoglobin</td>
<td>5805.0</td>
<td>11.61</td>
<td>876.9</td>
<td>75.1</td>
<td>55.3</td>
</tr>
<tr>
<td></td>
<td>Myoglobin</td>
<td>1867.5</td>
<td>3.74</td>
<td>252.1</td>
<td>24.16</td>
<td>17.79</td>
</tr>
<tr>
<td></td>
<td>Cytochrome ( c )§</td>
<td>16.6</td>
<td>0.033</td>
<td>2.51</td>
<td>0.215</td>
<td>0.158</td>
</tr>
<tr>
<td>Horse; 455 kilos, ( S ) 6.23 sq.m., ( M ) 205 kilos</td>
<td>Myoglobin</td>
<td>1345.2</td>
<td>2.96</td>
<td>215.9</td>
<td>18.50</td>
<td>13.67</td>
</tr>
<tr>
<td></td>
<td>Cytochrome ( c )§</td>
<td>24.3</td>
<td>0.053</td>
<td>3.90</td>
<td>0.334</td>
<td>0.247</td>
</tr>
</tbody>
</table>

* For concentration of chromoproteins see Tables I to III.
† From data of Crandall and Drabkin (13).
‡ Values based on complete analyses of individual organs.
§ Values based on (total muscle cytochrome \( c \)) 0.8. In the dog, similar values were derived from (total cytochrome \( c \) in muscles, heart, liver, and kidneys) 0.93.
∥ Based on a concentration of 16.3 gm. per 100 ml. of blood (34).
¶ Owing to variations in the myoglobin content, a value up to 41 gm. could be calculated for the total myoglobin of an adult man of 70 kilos.
** Steeplechase thoroughbred, out of training.
(Column 4, Table IV) discloses that, in the case of hemoglobin, closely similar values (mean = $12.7 \pm 0.7$ gm. of hemoglobin per kilo of body mass) are obtained in the different species. Hence, the content of hemoglobin is directly proportional to body mass.

3. Columns 5, 6 and 7 of Table IV contain the values of chromoprotein content, calculated respectively per sq. m. of body surface area and per fractional exponent of the body mass, $W^{0.7}_{kg}$, $W^{0.75}_{kg}$, and $W^{0.67}_{kg}$ (2, 5) and $W^{0.67}_{ti}$ (3). An examination of the values in these columns makes it clear that a similar, significant deduction may be made by the use of any one of the three bases of reference, namely that the cytochrome $c$ content is, in four of the five species (rat, dog, man, and cow), quite constant either per sq. m of estimated body surface or per fractional exponent of the body weight. Therefore, the total content of cytochrome $c$ is proportional to an exponential function of the body mass, close to $W^{0.67}$, which is theoretical for the relationship of surface to volume. However, an extension of this deduction as a generalization to all mammalian species is prohibited, since the horse is exceptional. The body of the horse appears to contain too much cytochrome $c$ in comparison with those of the other species examined.

DISCUSSION

The relationships of hemoglobin and cytochrome $c$ to body mass, though different, are equally remarkable. The finding that the total content of hemoglobin in different species is directly proportional to their body size seems to be consonant with the oxygen transport function of this chemical. The size of biological transport systems, functioning spatially, is apparently accommodated to the dimensions of the space they supply. Calculations, made by the writer (12) a number of years ago, from available information on the size of the mammalian erythrocyte, its water content, and the molecular volume of hemoglobin (based on dimensional data of Perutz (35)), indicated that the intracellular concentration of hemoglobin of the order of 34 gm. per 100 ml. is close to the maximum possible, under normal conditions. This must have consequences. An increase in hemoglobin above normal levels would demand either a change in the nature of the red blood cells or an increase in their number. Also, normally, owing to rough similarity in the size of the erythrocytes and in the relative volume of cells to plasma in different mammals, the relation of proportionality of hemoglobin content to body mass may be expected to apply to that of erythrocyte content and blood volume.

Though not applicable to all species, the proportionality of cytochrome $c$ content to surface area or to a fractional exponent of the body mass, as $W^{0.7}_{kg}$, is, nevertheless, one of the most striking relationships thus far uncovered for a cellular chemical. The involvement of cytochrome $c$ in
the mechanics of cellular oxygen use lends especial cogency to the above relationships, since the basal energy metabolism as well as the basal rate of oxygen consumption is also proportional in different species to their surface areas or to $W_{kg}^{0.7}$.

The chromoprotein-body mass relations, implied by the data in Table IV, may be expressed mathematically by so called heterogonic equations (equations expressing "degree of disproportionality" or of "similitude" (2, 3, 36)) of the general form $Y = aX^n$ or $a = Y/X^n$. In such an equation $Y$ and $X$ represent two separate quantities or rates, which may be disproportional to each other, but for which similitude can be obtained by means of the constants $a$ and $n$. In the present work, $Y$ is the quantity of chromoprotein in gm. and $X^n$ is the body mass in kilos, raised to an appropriate power, $n$. In the case of hemoglobin (Column 4, Table IV) $n$ is obviously close to unity and $X^n$ may be substituted by $W_{kg}$, while $a = 12.7$ gm., the mean of the values in Column 4. Thus, we may write

$$\frac{Hb}{W_{kg}} = 12.7 \text{ gm.}$$  \hspace{1cm} (1)

In the case of cytochrome c (Column 6, Table IV) $n = 0.7$ and $X^n$ may be replaced by $W_{kg}^{0.7}$, while $a = 0.039$ gm., the mean of the values in Column 6 for rat, dog, man, and cow. For the horse, $a = 0.275$ gm., an appreciably higher value. The relationships may be written

$$\frac{\text{Cytochrome c (rat, dog, man, cow)}}{W_{kg}^{0.7}} = 0.039 \text{ gm. or } 3.0 \mu M$$ \hspace{1cm} (2)

$$\frac{\text{Cytochrome c (horse)}}{W_{kg}^{0.7}} = 0.275 \text{ gm. or } 21.2 \mu M$$ \hspace{1cm} (3)

From pertinent data in the literature on the basal metabolic rates of the rat, dog, man, cow, and horse, the writer has obtained the following relation:

$$\frac{\text{Basal metabolic rate}}{W_{kg}} = 86.5 \text{ kilocalories per day or } 0.001 \text{ kilocalorie per sec.}$$  \hspace{1cm} (4)

The thermal equivalent of oxygen is 4.825 kilocalories per liter at the average basal R.Q. of 0.82. With this factor, equation (4) may be modified to

$$\frac{\text{Basal rate of oxygen consumption}}{W_{kg}^{0.7}} = 0.207 \text{ ml. per sec. or } 9.2 \mu M \text{ per sec.}$$  \hspace{1cm} (5)

Although it may not be strictly correct to talk about a "turnover rate" for cytochrome c, equations (2), (3), and (5) establish empirical relation-
ships between the rate of oxygen consumption and the quantity of this chromoprotein. In the rat, dog, man, and cow, the relationship is the same, namely 3 μM of cytochrome c to 9 μM of O₂ per second (basal state), whereas in the horse it is 21 μM of cytochrome c to 9 μM of O₂ per second (basal state). Adolph (36) has used the data on hemoglobin, myoglobin, and cytochrome c content in the writer’s preliminary report (1), and has chosen to lump together the data on the cytochrome c of the horse with that of the other species by the device of appropriately raising the value of n in the heterogonic equation. This is mathematically permissible, and suggests that rough similitude is possible for all the species. However, at this stage, it appears more objective to accept and deal with, rather than to mask, the dissimilarity of the horse.

It may be pointed out that the similarity of energy metabolism and oxygen consumption per W₉, in different species is regarded as applicable only to measurements in the basal state. However, it seems improbable that the relationship of cytochrome c content to the same base has similar connotations. Attention is again called to the fact that 80 per cent of the total cytochrome c is in skeletal muscle, the tissue of periodic, mechanical work. W₉, is thought to represent that fraction of the body mass which is “metabolically effective” (2, 3). It becomes pertinent to inquire to what extent the skeletal muscles (which form 36 to 42 per cent of the body weight), and the cytochrome c contained therein, participate in the basal metabolism and oxygen consumption. To answer this question, the writer has assembled in Table V reliable recent data, obtained by means of vascular catheterization (30, 37-40), upon the basal metabolism of individual human organs. When the separate data on brain, heart, kidneys, and liver, which may be regarded as organs of continuous metabolic activity, are brought together and summated, an important deduction becomes inescapable. In the basal state, these organs, which comprise only some 5 per cent of the body mass, take some 70 per cent of the total blood flow and are responsible for 70 per cent of the metabolism (i.e. oxygen consumption). Clearly, shifts in the allocation of metabolic work (as well as in metabolic pattern) must occur in the periodic changes from a basal or resting to a working state, involving muscular activity. Skeletal muscle is known to be supplied with a unique capillary bed, which can open up to allow large increases in blood flow, and, hence, indirectly in oxygen consumption. In the basal state, the skeletal muscle is truly quite inactive metabolically, and this large fraction of the body mass is responsible for only 15 per cent or less of the total metabolic activity. However, the relative contribution of muscle tissue to metabolism becomes appreciably greater when mechanical work is done. This can only mean that “effective metabolic mass” is of very different magnitude
TABLE V

Allocation of Total Blood Flow (Cardiac Output) and Oxygen Consumption in Man in Basal State

Organs which form about 5 per cent of the total body mass are responsible for 70 per cent of the basal metabolism and take about 70 per cent of the cardiac output.

<table>
<thead>
<tr>
<th>Tissue or organ*</th>
<th>Per cent of body mass of 70 kilos</th>
<th>Blood flow through organ</th>
<th>O₂ consumption by organ</th>
<th>Visceral blood flow, per cent of cardiac output (total flow) of 590 ml. per min.</th>
<th>Visceral O₂ consumption, per cent of total O₂ consumption of 248 ml. per min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>100.00</td>
<td>5390†</td>
<td>248.0</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Brain</td>
<td>2.00</td>
<td>756‡</td>
<td>47.6‡</td>
<td>14.03</td>
<td>19.2</td>
</tr>
<tr>
<td>Heart</td>
<td>0.47</td>
<td>215§</td>
<td>25.8§</td>
<td>3.99</td>
<td>10.4</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.47</td>
<td>1248‖</td>
<td>21.7‖</td>
<td>23.05</td>
<td>8.8</td>
</tr>
<tr>
<td>Liver</td>
<td>2.30</td>
<td>1683¶</td>
<td>70.7¶</td>
<td>21.21</td>
<td>32.1</td>
</tr>
<tr>
<td>Total, brain, heart, kidneys, liver</td>
<td>5.24</td>
<td>3002</td>
<td>174.8</td>
<td>72.3</td>
<td>70.5</td>
</tr>
<tr>
<td>Rest of body by difference</td>
<td>94.76</td>
<td>1488</td>
<td>73.2</td>
<td>27.7</td>
<td>29.5</td>
</tr>
<tr>
<td>Muscles</td>
<td>42.00</td>
<td>650**</td>
<td>32.4**</td>
<td>12.2</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>824††</td>
<td>40.6††</td>
<td>15.3</td>
<td>16.4</td>
</tr>
</tbody>
</table>

* For organ weights see Table II.
† Calculated from the basal oxygen consumption rate of 248 ml. per minute and an arterial venous difference in oxygen of 4.6 volumes per cent.
‡ Calculated from data of Kety and Schmidt (37).
§ Calculated from data of Bing et al. (30).
‖ Calculated from averages of data of Chasis et al., Bradley et al., and Clark et al. (38).
¶ Calculated from averages of data of Myers and Bradley et al. (39). The values for oxygen consumption and blood flow are not attributable exclusively to liver, but include some other splanchnic tissues.
** Calculation based on assumption that the metabolism of resting muscle is the same as that of the “remaining organs” (i.e. organs exclusive of brain, heart, kidney, and liver).
†† Calculation based on assumption that the metabolism of resting muscle is 20 per cent higher than that of the “remaining organs.” The value of 824 ml. per minute for blood flow through the skeletal muscle mass agrees well with that of 798 ml. per minute, based on the determination of Asmussen et al. (40) on the leg muscles of man.

in the basal and working states. Since 80 per cent of the cytochrome c is in skeletal muscle, one may conclude that only a fraction of the total cytochrome c activity is exhibited in the basal state, and that the total content of cytochrome c must be related to a factor such as metabolic ca-
pacity rather than basal metabolism. The metabolic capacity, evaluated from the rates of oxygen consumption during prolonged muscular work or at peak effort, possible only for brief intervals of time, is respectively 10- to 20-fold greater than the basal metabolism, i.e. an increase from 9 to 90 and 180 μM of O₂ per second. The finding of constancy of cytochrome c content per W²/kg in rat, dog, man, and cow suggests, therefore, that in these species there is a similar relationship of oxygen consumption capacity to basal oxygen consumption, which may be designated as the index of the expansibility of the metabolism. In these species the degree of expansibility of metabolism with muscular work should be the same, namely 10/1 to 20/1 (from the above relative values of O₂ consumption). Present information (2) does not allow an unequivocal conclusion that the horse has a greater metabolic capacity than the other species. However, of the five species examined, the horse is the most representative as an organism which does long continued, hard muscular work. Consonant with this, is not only the high cytochrome c content but also the very high myoglobin content of horse skeletal muscle. While the data suggest that the "turnover rate" with respect to oxygen and cytochrome c is lower in the horse than in the other species, the interpretation could also be made that in the horse there is a greater potentiality for an expansion of cytochrome c function, in the sense that less strain is placed during prolonged muscular work on the mechanism of oxygen utilization.

To return to oxygen transport, a further deduction of interest may be drawn from the data in Table V. The proportionality of the oxygen consumption of tissues with the rate of the blood flow through them suggests that the function of the carrier, hemoglobin, may be regarded as passive, the rate of oxygen supply being largely dependent on circulatory dynamics (cardiac rate, distribution of blood supply, etc.).

The data in Column 4, Table IV, upon myoglobin suggest a tendency for an actual increase in the content of this pigment per kilo of body weight with increasing size of species. Rough similitude for the myoglobin content in the different species can be shown from our data, as Adolph has pointed out (36), by the use of an exponent, n, greater than unity in the heterogonic equation. But, owing to the variability in the myoglobin content (dog and horse, Table IV), the establishment of a reliable value of n is not possible, and, in the case of this chromoprotein, stretching of the mathematical treatment to establish similarity would seem to be of questionable value.

The writer is indebted to Dr. D. K. Detweiler of the School of Veterinary Medicine, University of Pennsylvania, for supplying fresh tissues from cow and horse, and to the staffs of the Philadelphia General Hospital and the Hospital of the University of Pennsylvania for the human material.
SUMMARY

1. The total body content of hemoglobin, myoglobin, and cytochrome c has been determined in five species, rat, dog, man, cow, and horse, and an interspecies comparison has been made. The comparative data have disclosed significant relationships of these chromoproteins to body mass.

2. In all the species, the quantity of hemoglobin, the oxygen transport chemical, has been demonstrated to be relatively constant per kilo, and, hence, directly proportional to body mass. In the different species, the relationship is \( \text{Hb}/W_{kg}^{1.6} = 12.7 \text{ gm} \). The rate of oxygen supply appears to be largely dependent on circulatory factors, and the function of the oxygen carrier may be regarded as an essentially passive one.

3. The total content of cytochrome c, involved in cellular oxygen utilization, has been shown, on the other hand, to be proportional, in four of the five species, to the estimated body surface area or to \( W_{kg}^{0.7} \). The relationship, in rat, dog, man, and cow, is \( (\text{cytochrome c})/W_{kg}^{0.7} \sim 3.0 \mu\text{M} \). Correlated with the above, \( (\text{basal energy metabolism})/W_{kg}^{0.7} = 0.001 \) kilocalorie per second or 9.2 \( \mu\text{M} \) of \( \text{O}_2 \) per second, while \( (\text{metabolic capacity})/W_{kg}^{0.7} = 0.01 \) to 0.02 kilocalorie per second or 90 to 180 \( \mu\text{M} \) of \( \text{O}_2 \) per second. In the horse, the relationship of cytochrome c to body mass was found to be different from the other species, and was comparatively too high per \( W_{kg}^{0.7} \).

4. In all the species examined, the concentration of cytochrome c in cardiac muscle, a tissue of continuous work, was higher by a factor of 3 to 6 than in skeletal muscle, which is subject to periodic bursts of activity involving large energy expenditures. The myoglobin concentration, on the other hand, in two of the species (dog and horse), was higher in skeletal muscle than in heart.

5. The significance of the deductions drawn from the observations has been discussed. Attention has been directed to limitations in the commonly used basal metabolic state reference base, as well as in the concept of "metabolically effective body mass."

BIBLIOGRAPHY

THE DISTRIBUTION OF THE CHROMOPROTEINS, HEMOGLOBIN, MYOGLOBIN, AND CYTOCHROME c, IN THE TISSUES OF DIFFERENT SPECIES, AND THE RELATIONSHIP OF THE TOTAL CONTENT OF EACH CHROMOPROTEIN TO BODY MASS

David L. Drabkin and With the technical assistance of Priscilla Fourer, Jacqueline M. Fetsko, Anna May Dych, Charlotte Glauser, and Jean Randall


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