IN THE LABORATORY

The Specific Heat of Human Blood[1]

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Although the specific heat of blood was taken to be 0.90 by Stewart (*3*), no detailed report on its determination is available. In studying digital blood flows and pressures by the calorimetric method (*1*), with a view toward determining the effect of viscosity on vascular resistance in the living human subject, it was necessary to be sure that the specific heat of blood did not vary with erythrocyte concentrations such as are found in anemia and polycythemia.

A wide-mouthed Dewar flask, holding approximately 600 cc of fluid, was employed as a calorimeter. Into this flask, through a large cork stopper, were thrust a heating blade, a perpendicular hand stirrer, and a Beckman thermometer set at approximately 30–35° C. The heating device was connected in series to an ammeter and through an auto-transformer to an outlet of ordinary alternating current (110 v). The auto-transformer was regulated to deliver 0.5 amp constantly.

To determine the hydrothermic equivalent of the calorimeter, 595 gm of distilled water was placed in it, the current was turned on and adjusted to 0.5 amp, with a constant rate of stirring at approximately 150 strokes/min, and the rise in temperature per minute then determined. When a constant reading was obtained, the current was turned off and the fall in temperature per minute with the same rate of stirring was determined. In the formula, the rise and fall in temperature per minute were added, the heat of stirring being in this manner canceled out. Following the determination for water, a comparable volume of 1–25 molar solution of potassium carbonate with a known specific heat (0.76) was introduced into the calorimeter. With the current again adjusted to 0.5 amp, both the rise in temperature per minute and the fall in temperature after the current was turned off were determined. If x = the hydrothermic equivalent of the calorimeter, a = the rise in temperature per minute of water with the current on, b = the fall in temperature per minute of water with the current off, W_1 = the weight of water in grams, and K_1 = the specific heat of water at 30–35° C, (0.997), the number of calories delivered per minute would then be $(a+b)(K_1W_1+K_1x)$. Similarly, for the solution of known specific heat, if x = the hydrothermic equivalent of the calorimeter, c = the rise in temperature per minute for the solution with the current on, d = the fall in temperature per minute for the solution with the current off, K_2 = the specific heat of the solution, and W_2 = the weight in grams of the tested solution, then $(c+d)(K_2W_2+K_1x)$ represents the number of calories elaborated per minute in the solution.

Since the amount of heat elaborated for the water and the known solution is the same per minute, because the amperage in each case is 0.5 continuously, then $(a+b)(K_1W_1+K_1x) = (c+d)(K_2W_2+K_1x)$. Solving this equation,

$$x = \frac{K_2W_2(c+d) - K_1W_1(a+b)}{K_1(a+b-c-d)}.$$

The hydrothermic equivalent was thus determined to be 108. To calculate the error inherent in the method, 5 determinations of the specific heat of 1–25 molar potassium carbonate were made, using 108 as the hydrothermic equivalent. These were 0.74, 0.75, 0.76, 0.76, and 0.75, respectively, the maximum error being 3% and the average error less than 1%.

The specific heat of the blood was then determined. Clotting was prevented by the addition of 10 cc of 30% sodium citrate to each 600 cc of blood. In order to differentiate the specific heat of cells from that of plasma, one determination was made of plasma alone. This was done in the following manner: The number of calories elaborated by 0.5 amp was again determined for 595 gm of water. A comparable volume of plasma was then substituted for the water, and the total number of calories elaborated by the identical amperage determined. If K_3 = the specific heat of the plasma, e = the rise in temperature per minute in the calorimeter containing the plasma, with the current at 0.5 amp, f = the fall in temperature per minute after the current was turned off, W_3 = the weight of the plasma in grams, and W_c = the hydrothermic equivalent of the calorimeter (determined above), then $(e+f)(K_3W_3+K_1W_c)$ is the number of calories elaborated per minute in the sample of plasma. Since the calories elaborated in the water were again the same as those elaborated in the plasma, then $(a+b)(K_1W_1+K_1W_c) = (e+f)(K_3W_3+K_1W_c)$. Solving,

$$K_3 = \frac{K_1W_1(a+b) + K_1W_c(a+b-e-f)}{W_3(e+f)}.$$

The specific heat of normal blood plasma was found to be 0.94.

A second determination was made using a comparable volume of blood from which most of the plasma had been separated. The specific heat of this mixture of cells and plasma was determined in the same way as for plasma. The hematocrit was also determined in the standard manner, in a Wintrobe tube. The volumetric hematocrit was

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corrected for "trapped" plasma (4.5% by volume of cells) (*2*) and then converted into gravimetric percentage as follows: The specific gravity of plasma is 1.026 (average) and of whole blood containing 5,000,000 erythrocytes, 1.060 (*2*). The hematocrit of such blood is 43.2 by volume (*4*), corrected for "trapped" plasma to 41.3. If x = the specific gravity of cells, then $41.3x + (58.7)(1.026) = (100)(1.060)$. Solving, $x = 1.108$. If the volumetric hematocrit is h_1, and the gravimetric h_2, then

$$h_2 = 100\frac{1.108\,h_1}{1.108\,h_1 + 1.026(100 - h_1)}.$$

Solving,

$$h_2 = \frac{110.8\,h_1}{.082\,h_1 + 102.6}.$$

In calculating the specific heat of the cells, if x = the specific heat of the cells, K_3 = the determined specific heat of plasma, K_4 = the determined specific heat of the mixture of cells and plasma, and h_2 = the gravimetric hematocrit (corrected for "trapped" plasma) of this mixture in per cent, then $100\,K_4 = K_3(100 - h_2) + h_2x$.
Solving,

$$x = \frac{100\,(K_4 - K_3) + h_2K_3}{h_2}.$$

By this method the specific heat of cells, which may be redesignated K_5, was determined to be 0.77.

To test the accuracy of the method, the specific heats of several random mixtures of cells and plasma were calculated. If x = the specific heat of the mixture, and h_2 = the gravimetric hematocrit (corrected for "trapped" plasma) of that mixture, then $100\,x = (h_2 - 100)\,K_3 + h_2K_5$. The specific heat of this mixture was then measured directly and checked against the calculation. The results of four such calculations were as follows:

Sample No.	1	2	3	4
Hematocrit volumetric, uncorrected	32.4	42.4	41.6	30.0
Specific heat, calculated	0.89	0.87	0.87	0.89
Specific heat, actual	0.89	0.86	0.86	0.88

The small discrepancies between calculated and actual specific heats may be attributable to the error inherent in the method, and perhaps in part to minor variations in the hemoglobin content of the cells and in the percentage of solids, especially proteins, in the plasma. The correlation is, nevertheless, good, and the specific heat of any mixture of cells and plasma can be calculated from the hematocrit.

The low specific heat of cells in comparison with plasma is probably due to their greater concentration of solids, especially iron.

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Qualitative Reaction for 2,4-Dichlorophenoxyacetic Acid[1]

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There has arisen a need for a chemical method of identification of trace amounts of 2,4-D. Various bioassays employing plants have been proposed (*5*), as have methods of quantitative determination of the material, ranging from several gamma/ml to a gram and up (*1, 3, 4*). Bandurski (*1*) has employed a spectrophotometric method for the determination. However, quantitative methods of analysis are not always suitable for qualitative identification of a chemical.

To date, no satisfactory color derivative of 2,4-D has been developed that would serve as a quantitative means of identification (*5*). Various derivatives have been tried, including the nitro derivatives and their reduction analogs (*3*), but these have not proven satisfactory.

Certain characteristic salts (*3*) are formed by 2,4-D, the most distinctive of which is the uranyl salt. This salt is bi-axial in one plane with distinctive markings radiating pinnately from the axes. However, since the range of this method is 200–400 γ of material/ml, it is not capable of detecting really micro amounts.

Various means of identification have been tried in this laboratory with varying degrees of success. A halogen-substituted aromatic compound, if negatively substituted in the ortho or para position, will react with ammoniacal alcohol to form an amino derivative. The sodium-1-2-naphthoquinone-4-sulfonate will then detect the amino group (*2*). This reaction employing sodium-1-2-naphthoquinone-4-sulfonate was found to be reasonably effective in detecting 2,4-D, but lacked sensitivity and ease of application.

It was noted that when acid was warmed gently in concentrated sulfuric acid with 1,8-dihydroxy naphthalene 3,6-disulfonic acid (chromotropic acid), a characteristic wine-purple color resulted. As little as 0.05 γ of 2,4-D/ml could be detected by this test. As far as we are aware, this reaction has never been reported in the literature.

The procedure consists of introducing 2,4-D into a test tube and bringing it down to dryness. It has been found that benzene is the most suitable material for extraction of the 2,4-D for this reaction. Certain other solvents, notably alcohol, interfere slightly with the results. After bringing the material down to dryness, a few crystals of the chromotropic acid are introduced on the end of a micro spatula, 2 ml of concentrated sul-

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