THE SPECIFIC HEAT AND THE HEAT OF COMPRESSION OF HUMAN RED CELLS, SICKLED RED CELLS, AND PARACRYSTALLINE RAT RED CELLS*

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This paper is concerned with two thermal properties of red cells, paracrystalline red cells, and sickled red cells. The first is their specific heats, which, generally speaking, can be expected to decrease as the degree of molecular orientation increases. The second is the thermal effect of compression, heating or cooling occurring on the application of pressure according to whether the orderliness of the molecules is decreased or increased. Since there is evidence that the molecules of the mammalian red cell are arranged to form a structure, well defined at the surface and much less well defined in the interior (Ponder, 1951), an investigation of the specific heats and the effects of compression may give a certain amount of information about the molecular orderliness which exists, particularly in the case of cold paracrystalline rat red cells (Ponder, 1945) and sickled red cells.

There seems to be no doubt that the orderliness of the molecules in the cold paracrystalline rat red cell is greater than that in the normal red cell or in the paracrystalline rat red cell when warmed (when it ceases to be paracrystalline), for both birefringence and x-ray spacings corresponding to 58 A and 45 A have been found (Dervichian, Fournet, Guinier, and Ponder, 1952), the birefringence and the spacings disappearing when the cells are warmed and reappearing in the cold. The passage from the normal to the paracrystalline form is also accompanied by a small but definite decrease in the specific heat (Ponder, 1953). The situation is not so clear in the case of the birefringent (Ascendi and Silverstroni, 1953) sickled red cell. Perutz and Mitchison (1950) have shown that hemoglobin-S is relatively insoluble, and because sickled red cells give anomalous colors similar to the dichroism seen in crystals of a reduced hemoglobin, have concluded that deoxygenation results in the crystallization of hemoglobin-S inside the cell, and that sickling is the result partly of the habit of the crystals and partly of the loss of water which would be expected to accompany crystallization. They suggest that this water diffuses out of the cell and that this results in the surface structure collapsing; measurements of water content show, how-

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ever, that only about 2 per cent of the cell water is lost when sickling occurs. Opposed to the view that hemoglobin-S undergoes crystallization is the view that it undergoes gelation (Singer and Singer, 1953) with tactoid formation (Harris, 1950); this is compatible with the results of x-ray scattering, which certainly does not provide evidence of a crystalline structure.

1. Specific Heat

This can be measured by a method already described (Ponder, 1953), which determines the rate of cooling of a weighed mass of red cells, surrounding a series of thermopile junctions arranged so as to sample the temperature of the mass; the heat is lost to the walls of a large chamber containing air, the mass of cells, and the thermopile, and immersed in a water bath kept at about 2°. The specific heat of the human or the rat red cell in the normal state is 0.87 ± 0.02, but that of the paracrystalline rat red cell (obtained by keeping rat red cells for several days in cold sodium citrate) is 0.74 ± 0.02. The change from the higher to the lower value occurs in the neighborhood of 6°, the temperature near which, as judged by other criteria (resistance to lysis by cold water, appearance of birefringence, etc.) the paracrystalline state develops. No such decrease in specific heat occurs, between 1 and 15°, in the case of either human red cells or fresh rat red cells in saline or in plasma.

A new series of determinations has given 0.85 as the specific heat for both normal human red cells and sickled cells, all the observations falling within the range 0.82 to 0.88. The determinations were carried out on red cells in which the sickling was produced by a reduction of the O_2 tension, and not by the addition of reducing agents. There are a number of differences, not generally appreciated, between sickling which results from a lowered O_2 tension and that which is produced by the addition of metabisulfite, etc.

While the essential identity of the specific heats for normal and for sickled red cells does not preclude sickling being the result of a crystallization of the abnormal hemoglobin-S, it certainly gives no support to the idea. It is more compatible, on the other hand, with sickling being the result of gel formation.

2. The Thermal Effects of Compression

The method used is described here for the first time. Packed red cells are contained in a circular brass tube 14 mm. in diameter and 50 mm. long (Fig. 1). A circular flange, 40 mm. in diameter, is welded to the top of the tube. The cover of the tube is another circular flange 40 mm. in diameter, perforated by an opening leading to a brass tube with a screw attachment at its upper end which fits the outlet of the reducing valve of a N_2 cylinder. Three screws, passing through the flange and the cover, allow the latter to be held against the flange in a leak-proof manner, stopcock grease being applied to the opposing surfaces.

The thermopile is attached to a flat plate, 40 mm. long, which projects downwards from the cover. The 3 copper-constantan junctions lie near the lower end of the plate, the wires going to two reference junctions and to the two terminals, and passing through small holes in the cover. The sealing of these holes has pre-

sented great difficulty, but was finally accomplished by dipping the upper side of the cover in liquid lucite which was afterwards allowed to solidify. The galvanometer used was a moving-coil instrument with a resistance of 12 ohms and a sensitivity of 0.1 microvolt per mm. of scale deflection at 1 meter.

The tube is filled with water or with red cells packed into it by centrifuging. The galvanometer is shunted. The thermopile is inserted into the water or the red cell mass, and the cover is screwed down. Mineral oil is run into the long brass tube which is screwed to the outlet of the reducing valve of the N_2 cylinder. The apparatus is arranged so that the tube containing the cells and the thermopile hangs

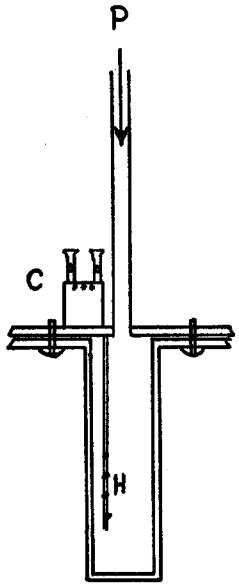

FIG. 1. Diagram of the chamber containing the water or red cells and the thermopile. *H*, the warm junctions, *C* the cold reference junctions, and *P* the tube through which the pressure is suddenly applied.

in a large water bath at 10°. After a period of equilibration, the galvanometer is unshunted and a pressure of 10 atmospheres is suddenly applied to the contents of the tube. The galvanometer deflection, which occurs almost at once, is observed. The pressure is then released by turning the screw connection attached to the reducing valve, and pressure can be reapplied as many times as may be necessary for the obtaining of a series of values for the thermal effect of compression.[1]

The results obtained can be summarized by saying that the heat of compression, which is positive in sign, is the same for water and for packed human or

[1] Repeated applications of pressure to packed red cells give substantially the same heat of compression. There is therefore no indication that the application of pressure produces irreversible changes in molecular arrangement.

rat red cells at 10°. The heat obtained with paracrystalline rat red cells, on the other hand, is about 25 per cent less. Since heat is released on the application of pressure, $(dT/dP)_s$ for water,[2] normal cells, and sickled cells (see, however, footnote 3) $>$ $(dT/dP)_s$ for paracrystalline cells, and > 0, or, to state the result another way by using one of Maxwell's relations, $(dV/dS)_p$ for water, normal cells, and sickled cells $>$ $(dV/dS)_p$ for paracrystalline cells, and > 0. Here S is entropy, p pressure, V volume, and T the absolute temperature. In terms of the reciprocals of these derivatives, $(dS/dV)_p$ for paracrystalline cells $>$ $(dS/dV)_p$ for water, normal cells, and sickled cells, and > 0. Since an increase in entropy corresponds to a decrease in order, a positive value of $(dS/dV)_p$ means that the system becomes less ordered by isobaric expansion and more ordered by isobaric compression. This seems to be the case for all three kinds of cell, but the order in the paracrystalline structure is less diminished by compression than the order in the normal and the sickled cell. This result is not unexpected, but unfortunately it is not possible to analyze it further because of the number of unknowns involved.

The important point is that the heat of compression of packed sickled cells is the same, to within 5 per cent, as that of normal red cells;[3] *i.e.*, this method

[2] The heat of compression of water is given by the expression

$$\Delta T = \frac{avT}{c_p} \cdot \Delta p$$

in which T is the absolute temperature, a the volume coefficient of thermal expansion at constant pressure p, v the volume per unit mass, and c_p the specific heat at constant temperature. ΔT changes sign at 4°C., where a changes sign; above 4°C., ΔT is positive. At a temperature of 10°C. and for an applied pressure of 10 atm., the value of ΔT is 0.0062°C., and the mean galvanometer deflection observed under the conditions of these experiments is 18.2 mm. This is within 0.2 mm. of the theoretical value obtained (by interpolation) from the values given by Lorentz (1927). In the case of the paracrystalline red cells, the mean value was 13.8 mm.; *i.e.*, 75.5 per cent of the value for water. In the case of sickled red cells, the mean value was 19.1 mm.; *i.e.*, 5 per cent greater (see footnote 3) than that for water. The maximum deflection is reached in from 2 to 3 seconds, and remains unaltered at least for 5 seconds. For practical reasons (to avoid blowing out the seating of the reducing valve, an accident which often happened in the earlier experiments) the pressure was not applied for longer periods; under any circumstance, however, ΔT would tend to fall from its maximum value as heat diffused from the chamber containing the thermopile towards the surrounding water bath. As regards reproducibility, see footnote 1.

[3] Very frequently, the heat of compression of sickled red cells is about 5 per cent greater than that of water or of normal red cells. Too much emphasis ought not to be placed on this, because the error of the method is itself about 5 per cent. Nevertheless, it is permissible to point out that the random breaking of links in a branched gel might result in a small increase in the heat of compression.

also fails to provide evidence for sickling being the result of crystallization of hemoglobin.[4]

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SUMMARY

The investigation of two thermal properties of red cells throws some light on whether sickling is a process involving the crystallization of a relatively insoluble hemoglobin. These properties are the specific heat and the heat of compression, both of which would be expected to become numerically less if the hemoglobin of the red cell were to crystallize. In the case of paracrystalline rat red cells, which give spacings at 45 A and 58 A by x-ray diffraction, the specific heat is reduced to 85 per cent of that of the normal red cells, and the heat of compression is only about 75 per cent of that found for the normal red cell. In the case of the red cell sickled by a reduction of the O_2 tension, the specific heat and the heat of compression are substantially the same as found for the normal red cell. This is an argument against sickling being the result of a crystallization process, and supports the observation that sickled cells do not give x-ray spacings. The result is compatible, on the other hand, with sickling being the result of the formation of an oriented and birefringent gel.

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[4] There is another possibility which cannot be disposed of at present. Dervichian has suggested, in a summary of a report presented to the 5th International Transfusion Congress (1954) that sickling involves a co-precipitation of Hb and lipids but only in the surface regions of the cell, the greater part of the Hb being unaffected. This might account for the absence of changes in the x-ray scattering, in the specific heat, and in the heat of compression. The point which presents the difficulty is the birefringence of the sickled cell. The birefringence found by Ascendi and Silverstroni is 0.0037, and falls within the range of the birefringences of other biological objects; it results, however, from taking the entire thickness of the cell, 2μ, as giving rise to the birefringence, and the latter would have to be much greater, *i.e.* outside the range found for other biological objects, if it arose only from regions situated at the surface. It is nevertheless possible that a co-precipitate of Hb and lipids might have a birefringence greater than that usually met with in the case of biological objects such as muscle, bone, etc.

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