A QUANTITATIVE STUDY OF THE FRAGMENTATION AND HAEMOLYSIS OF MAMMALIAN RED CELLS BY HEAT

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(With Three Text-figures)

This paper is concerned with quantitative aspects of the fragmentation of red cells by heat and of the haemolysis which accompanies it. The former process involves a splitting of the cell into fragments which retain, at least in part, some of the properties of the intact cell (ability to swell in hypotonic media, ability to undergo certain shape transformations, etc.; see Ponder, 1949). The latter process results in the formation of ghosts with properties which vary with the haemolytic system; in some systems, the cell becomes a ghost by an all-or-none process (as in hypotonic systems containing cells in low volume centration, see Parpart, 1931), while in other systems the ghosts are partially haemoglobinized and partially rigid. To describe the phenomena adequately, it is accordingly necessary to consider the transition of the red cell to the ghost as a process which can occur in several ways in addition to the all-or-none escape of Hb and the replacement of the cell by an unsubstantial ghost; it is all the more important to do this because evidence is accumulating that red cell ghosts in several kinds of haemolytic system possess both shape and rigidity (Ponder, 1942, 1950, Lindemann, 1949 a, b).

METHOD

The water-bath used for heating the cells consists of a highly insulated glass tank fitted with an immersion heater, an adjustable thermoregulator, and a stirring motor; another motor moves a rack-like tube holder to and fro in the water with an intermittent, jerky, motion at the rate of about 100 excursions of 1 cm. each per minute. Small glass tubes (75 x 10 mm.) contain 0.5 ml. of the blood or suspension to be heated; when the tubes are in place in the tube holder, the blood or suspension is immersed about 6 cm. below the surface of the water. The temperature of the bath can be kept constant to 0.05° C. by adjusting the thermoregulator.*

When the temperature of the bath has reached a selected temperature, e.g. 56° C., four to six small tubes containing the blood or suspension in which fragmentation is to be studied at that temperature are placed in the tube holder, and the shaking motor is started. After various times (3, 5 min., etc.) one of the tubes is removed and placed in an ice-bath for a few moments; cell counts and other determinations are then made on the contents of the tubes with as little delay as possible.

* The shaking mechanism was made for me by Mr Paul Cutajar of New York University Machine Shop. A convenient form of thermoregulator is one of a bimetallic type which can be quickly adjusted to any temperature over a wide range, and which operates without a relay.
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The bath is now allowed to cool to some second desired temperature, say 54° C.; the thermoregulator is set for this new temperature, and four to six tubes containing the blood or suspension are again placed in the tube holder and are heated with shaking for selected times before removal to the ice-bath. The process is repeated at lower temperatures (52, 50° C., etc.), and in this way one obtains a series of systems in which the red cells have been exposed, with constant shaking, to various temperatures for various times.

A small sample of each system is drawn up in a red cell counting pipette, and the cells plus fragments on both sides of a haemacytometer chamber (Spencer Brightline) are counted. When fragmentation is extensive, the sample is diluted appropriately so that about 800 cells plus fragments are counted; the coefficient of variation associated with the imperfect distribution of this number is ±3·5 %. So as to allow time for the smaller fragments to settle, the preparation is allowed to stand for about 5 min. in the counting chamber before counting is started. In order to avoid including ghosts in the count, the illumination is kept high, but, as will be seen below, there is often a question as to whether an object is a ghost or not, since haemolysis in heated systems is often not all-or-none. In some cases it is possible to count cells and fragments on the one hand and ghosts and incompletely haemoglobinized cells on the other; considerable judgement enters into making the counts, and the precision with which the same result can be obtained repeatedly on the same material increases with experience.

A sample of each system is transferred to a haematocrit tube (100 mm. with 1 mm. bore, sealed at the lower end), and is spun at 10,000 r.p.m. for 30 min. The volume concentration of the cells, fragments, and ghosts is measured in the usual way; the column of clear fluid overlying the packed mass is then removed, and the concentration of Hb in it is found photometrically and is expressed as a fraction of the concentration of Hb in the completely haemolysed system. If the former concentration, as a fraction of the latter, is \( C \), the quantity of Hb present in the column of clear fluid above the packed cells, fragments, and ghosts is \( p = C(1 - \rho_1) \), where \( \rho_1 \) is the volume concentration of the cells, fragments, and ghosts.

The upper part of the haematocrit tube, from which the clear Hb-stained fluid has been removed, is rinsed with saline introduced with a capillary pipette; this removes excess Hb from the walls of the tube. The haematocrit tube is then turned upside down and a one-hole rubber stopper is fitted over its closed end; supported in this stopper, it is suspended in a 100 × 13 mm. test-tube containing 1 ml. of water, and is spun slowly in the centrifuge. The spinning drives the cells, fragments, and ghosts from the inverted haematocrit tube into the water, where they haemolyse. The concentration of Hb in the solution which results is found photometrically, and is expressed as a fraction of the Hb concentration found by treating an unheated system similarly. If this concentration is \( C' \), the mean corpuscular Hb concentration of the objects in the packed column is \( C' \rho_0 / \rho_1 \), where the volume concentration of the cells in the unheated system is \( \rho_0 \).
(1) \textit{Fragmentation and the accompanying haemolysis}

The principal difficulty in the study of the kinetics of fragmentation is that haemolysis usually accompanies it, and that the haemolysis, unlike that in many haemolytic systems, is not necessarily all-or-none. This introduces severe limitations on the extent to which the phenomena can be dealt with quantitatively, and it will be convenient at the outset to see what these limitations are.

When a red cell, or the fragments into which a red cell breaks, loses a fraction $a$ of its initial Hb, its volume may or may not decrease in proportion. Let the change in volume with change of $a$ be $\frac{dv}{da} = k$; then $v = 1 - ka$ if the initial volume is denoted by unity. The amount of Hb which the cell loses is $a$, and this appears in the supernatant fluid of the system; the fraction $(1 - a)$ is left in the cell, and the corpuscular Hb concentration is always $(1 - a)/(1 - ka)$. In the case of individual cells, there are at least four possibilities to be considered.

\textbf{Case 1.} Complete loss of Hb, complete loss of volume; all-or-none haemolysis. Here $a = 1, k = 1$.

\textbf{Case 2.} Complete loss of Hb, no loss of volume; rigid ghosts. Here $a = 1, k = 0$. The corpuscular Hb finally becomes the same in concentration as that in the fluid surrounding the cells.

\textbf{Case 3.} Partial loss of Hb, no loss of volume; partial haemolysis or retention of Hb depending on whether the process is looked at from the standpoint of the cell or from that of the ghost. Here $a < 1, k = 0$. The corpuscular Hb concentration never becomes as low as that in the surrounding fluid.

\textbf{Case 4.} Partial loss of Hb, accompanied by loss of volume; small haemoglobinized ghosts. Here $a < 1$, and $k$ can have values between 1 and 0. When $k = 1$, the corpuscular Hb concentration remains unity throughout the process; when $k < 1 > 0$, the corpuscular Hb concentration is less than unity, but never becomes as small as that in the surrounding medium.

When observations are made on populations of red cells before and after heating, the results cannot always be reduced to one or another of these simple cases. In such systems we observe $\rho$, the fraction of the total Hb liberated into the supernatant fluid obtained by packing cells, fragments, and ghosts, and also $\rho$, the volume concentration of these packed objects. In place of the corpuscular Hb concentration, we now have the mean corpuscular Hb concentration of the cells, fragments, and ghosts; this is $C_m = (1 - \rho)/(1 - ka)$, and the change in volume with loss of Hb is now $\frac{dp}{d\rho} = k$. Using these values, an attempt can be made to reduce the observations to one or another of four cases which correspond to those enumerated above.

\textbf{Case 1a.} Complete loss of Hb, complete loss of volume; all-or-none haemolysis. Plotting $\rho$ against $\rho$ gives a straight line with a slope of $-\rho_0$, which makes intercepts $\rho = \rho_0$ and $\rho = 1$. Determinations of $C_m$ show that it has the value of unity for all values of $\rho$. This case is illustrated by the behaviour of human red cells when heated to increasing temperatures or for increasing times (Fig. 1, 1a). Haemolysis as measured by $\rho$ increases and $\rho$ decreases linearly with it, complete haemolysis corresponding to a negligible volume of ghosts.
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Case 2a. Complete loss of Hb, no loss of volume; rigid ghosts. Plotting $\rho$ against $p$ gives a straight line parallel to the $p$-axis, i.e. with a slope of zero, and terminating in a point the co-ordinates of which are $\rho = \rho_0$, $p = 1 - \rho_0$. This is the largest value of $p$ which can occur in the system, because it is the value which makes the concentrations of Hb inside and outside the ghosts equal. When determined at this value of $p$, $C_m = \rho$, and this is the lowest value it can reach. Geometrically, the co-ordinates of the terminal point form a square with the $p$-axis and the vertical at $p = 1$ (Fig. 1, 2a). There is no example of this process occurring in heated systems as affecting all of the cells, but it is met with as a part of case 5, below.

![Graphs 1a-6a](image)

Fig. 1. To illustrate various possible relations between $\rho$ and $p$; for description, see text. Experimental results are shown as circles. 1a, washed human red cells; 5a, washed sheep red cells; 6a, washed human red cells, lecithinated. Other cases (2a, 3a, 4a) not met with experimentally in this investigation.

Case 3a. Partial loss of Hb, no loss of volume; partial haemolysis or retention of Hb. Plotting $\rho$ against $p$ gives a straight line parallel to the $p$-axis, but terminating in a point for which $p$ is less than $1 - \rho_0$. A rectangle, and not a square, is formed by the co-ordinates of the point, the $p$-axis, and the vertical at $p = 1$. The concentration of Hb inside the ghost never falls as low as that in the surrounding medium, and $C_m$ is always greater than $\rho_0$ (Fig. 1, 3a). There is no example of this process occurring in heated systems as affecting all of the cells, but, like case 2a, it enters into the description of compound cases, below.

Case 4a. Partial loss of Hb, accompanied by loss of volume; small haemoglobinized ghosts. Plotting $\rho$ against $p$ gives a straight line with a slope between zero and $-\rho$, ending in a terminal point with co-ordinates $\rho_1$ and $1 - \rho_1$, where $\rho_1$
is the (diminished) volume of the ghosts. $C_m$ determined at this value of $p$ exceeds $p_1$ but is less than unity, so that the concentration of Hb in the ghost never falls to that in the surrounding medium, and may be considerably above it (Fig. 1, 4a). Like cases 2a and 3a, there is no example of this process as affecting all the cells of a heated system.

In experiments with populations of cells, complex cases are met with over and above those which arise in the case of single cells. If there are $N$ cells in the system and if each can lose a fraction $a$ of its Hb, the Hb lost is $p = Na$, but the same value of $p$ can result from some of the cells having lost all of their Hb or from all of the cells having lost some of their Hb. The situation in the partially haemolysed system can thus be represented, generally, by

$$p = N_1a_1 + N_2a_2 + N_3a_3,$$

(1)

where $N_1, N_2, N_3$, etc., are fractions of the total number $N$ which behave in different ways as regards the loss of their pigment, as indicated by the different values of $a$. Each fraction may also behave differently as regards the loss of its volume, and so a large number of possible situations arise. Two of these are of particular interest in connexion with heated systems.

Case 5a. There are only two classes, $N_1$, the cells which haemolyse, and $N_2 = (N - N_1)$, those which remain intact. For the latter, $a_2 = 0$, and for the former $a_1 = 1$, $k = 0$. The value of $p$ increases because the value of $N_1$ increases, more and more cells losing all of their Hb to give the increase in $p$. This case is to be contrasted with case 2a, in which all the cells lose more and more of their Hb as $p$ increases. To distinguish between them we have to fall back on other criteria; when all the cells lose part of their Hb, the column of packed objects becomes uniformly paler as $p$ increases, but when some of the cells lose all their Hb, the remainder losing none, the column of packed objects is likely to show a contrast between haemoglobinized intact cells and pale ghosts, a decrease in the volume concentration of the cells being accompanied by an increase in the volume concentration of the ghosts. This is illustrated by the behaviour of rabbit or sheep red cells on heating, and shown in Fig. 1, 5a. Further, in case 2a, examination under the microscope shows more or less uniform objects which become paler as $p$ increases, whereas in case 5a, the field shows two categories of object, a fully haemoglobinized cell and a pale but voluminous ghost.

Case 6a. Again there are only two classes, $N_1$, the cells which haemolyse, and $(N - N_1)$, those which remain intact, but for the former $a_1$ and $k_1$ have values between 1 and 0, i.e. some cells haemolyse in a partial manner in Barón’s (1928) sense of the term. In this case the value of $p$ increases both because $N_1$ increases and because $a_1$ increases, and is always greater than can be accounted for by the diminution in $p$, so that plotting $p$ against $p$ gives a line such as that shown in Fig. 1, 6a (experimental data derived from heated human red cells treated with lecithin). Examination of the preparation with the microscope shows intact cells, decreasing in number as $p$ increases, together with ghosts and fragments with varying degrees of haemoglobinization.
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Finally, the best measure of the average number of fragments which each fragmenting red cell breaks into is

\[ f = \frac{N_f/N}{1 - N_{a=1, k=1}} \]  

(2)

where \( N \) is the initial number of cells per unit volume as seen on the counting chamber, \( N_f \) the number of cells, fragments, and visible ghosts (possessing volume and also haemoglobin, whether by reason of its being 'surplus Hb.' or merely 'equilibrium Hb' trapped in a voluminous ghost which has undergone some shrinking), and where \( N_{a=1, k=1} \) is the fraction of \( N \) which undergoes all-or-none haemolysis, e.g. which leaves no ghosts. If haemolysis is all-or-none, \( N_{a=1, k=1} \) is measured by \( p \). If haemolysis is of the type described by cases 2a, 3a, 4a, 5a or 6a, \( N_{a=1, k=1} \) is zero, and \( f \) is simply \( N_f/N \). In experiments with populations of red cells, situations may arise in which it is very difficult or impossible to determine what value ought to be given to \( N_{a=1, k=1} \), but no expression more simple than expression (2) seems to be a consistent measure of the amount of fragmentation. Fortunately, most systems in which fragmentation is produced by heat are examples either of case 1a or of case 5a.*

(2) Fragmentation as a function of time and temperature

Fragmentation as measured by \( f \) in expression (2) increases with the time during which the cells are kept at any one temperature, and increases as the temperature is increased. Fig. 2 shows a series of curves for human red cells, \( f \) being plotted against \( t \) at three different temperatures. The precision of the points, which is none too great under any circumstances because of the errors associated with the counting, is still further reduced by the heating of the cells not being instantaneous; it requires a short time for the systems in the small tubes to reach the temperature of the waterbath, and so the real value of \( t \) is always smaller than the recorded value. An attempt has been made to compensate for this by subtracting from all values of \( t \) the time required for the cells to reach 48° C., the temperature in the neighbourhood of which fragmentation begins; these small corrections affect the shorter times more than they do the longer ones.

At any one temperature, the relation between \( t \) and \( f \) seems to be a curve concave to the \( t \)-axis and proceeding towards an asymptote at \( t = \infty \). A situation in which some of the cells fragment before others would tend to give rise to a sigmoid relation between \( t \) and \( f \), but there are several secondary effects which obscure such a relation, e.g. a tendency for the more easily fragmented cells to break into a larger number of fragments than are derived from the cells which fragment with more difficulty. Perhaps for this reason, no evidence of a sigmoid relation has been obtained. As the situation stands, there is nothing to be gained by attempting to find an expression

* Increases in the volume of intact red cells as a result of heating have not been considered in the foregoing discussion. In some systems these are not only noticeable, but troublesome, systems containing heated cells giving greater values of \( p \) than otherwise identical systems containing unheated cells, even when some lysis occurs in the heated systems. This effect can be minimized by using as \( p_0 \) the volume concentration for a system which has been heated to a temperature a few degrees below \( T_a, p \), and then plotting \( p \) against \( p \), as above.
which will fit the curves, since by analogy with the haemolytic process, this expression
would almost certainly have to take account of a variation in resistance to fragmenta-
tion, of the number of fragments into which a cell of given resistance breaks, and
also of the velocity of whatever 'fundamental reaction' underlies the fragmentation.

The values of $f$ and of $p$ corresponding to the asymptotes, on the other hand, are
related to the temperature $T$ in a simple way. Call the particular values of $f$ and $p$
which correspond to the asymptotes $F$ and $P$; plotting them against the temperature
$T$ gives two good straight lines (Fig. 2, inset) with slopes $K_F$ and $K_P$, making

\[ F = K_F(T - T_0, F), \]
\[ P = K_P(T - T_0, P). \]

By means of these expressions, the fragmentation and haemolysis which occur at
the end of long times of heating (15 min. being considered as indefinitely long in
these experiments) can be expressed in terms of four constants, two for each process.
The temperature $T_0, F$ is the highest temperature to which the system can be heated
without fragmentation occurring, and the temperature $T_0, P$ is the highest tempera-
ture to which the system can be heated without haemolysis occurring; both values
are obtained by extrapolation. In the case of washed human red cells, $K_F = 1.22,$
$T_0, F = 49^\circ$ C., $K_P = 0.15, \text{ and } T_0, P = 49.6^\circ$ C. Extrapolation also gives a tempera-
ture at which $P = 1.0$, i.e. at which complete haemolysis occurs after 15 min. heating;
in the case of washed human red cells, this temperature is about $56^\circ$ C. It would
probably be a mistake, however, to attach much significance to this value or to the
corresponding value of $F (= 0.6)$, because it is doubtful whether the relation between
$F$ and $T$ and between $P$ and $T$ remains linear when the value of $P$ is greater than
about 0.7.

(3) Fragmentation of human red cells by heat; experimental
modifications of the process

The types of fragmentation which occur in preparations of human red cells in
plasma have already been described as being of three kinds (Ponder, 1949): the
appearance of radial defects from a central thinned-out area, with the subsequent
breaking of the cell into fragments bounded by these defects, the development of
irregularities at the cell margin and the fragmentation of the cell as a result of the
extension of defects originating from the bases of these irregularities, and a ‘beading’
of the cell rim with the development of fragments from the ‘beads’. The fragmentation
of washed human red cells occurs in essentially similar ways, except when the
cells are rendered spherical by being placed between slide and cover-glass; under
these conditions they become spherical and haemolyse without fragmenting as the
temperature is raised.

The three principal conclusions of this investigation can be illustrated by the
comparison, in a quantitative way, of heated systems containing (1) fresh human red
cells, freshly washed, (2) human red cells which have been kept at 4°C for several days
and then washed, (3) human red cells, washed and then treated with distearyl lecithin,
and (4) fresh human red cells in plasma. The results illustrate the conclusions that
heating under different conditions may give rise to ghosts with different properties
(comparison of 1 with 3), that conditions which tend to render the cells spherical
prevent its fragmentation (comparison of 1 with 2 and 3), and that plasma contains
substances which inhibit both fragmentation and heat haemolysis (comparison of
1 with 4).

(i) Fresh human red cells, freshly washed. In this system, haemolysis is all-or-none
(Fig. 1, 1a), and so $F$ can be found from expression (2) with $N_{\text{a}=1, k=1}=P$. The
straight line which results is shown in Fig. 3 (man, case 1a), together with the
experimental relations from which it is derived, these being a linear relation between
$P$ and $T$ and a relation between $N_{F}/N$ and $T$, the latter passing through a maximum.
In this system, fragmentation begins at a lower temperature than that at which
haemolysis begins, and at a temperature such as 53°C the number of objects seen
on the counting chamber is about three times the initial number; all of these,
moreover, are cells or haemoglobinized fragments ($C_m = 1$ for all values of $P$), the
ghosts being invisible under the conditions of illumination used.

(ii) Human red cells, kept at 4°C for 48 hr.; then washed. Again haemolysis is
all-or-none, and $F$ is found as in the preceding case. The keeping of the cells for
48 hr. at 4°C, however, enhances the haemolytic aspect of the heating effect, so
that the relation between $P$ and $T$ is represented by very nearly the same straight
line (although on a different scale) as that which represents the relation between
$F$ and $T$ (Fig. 3, man, 48 hr. at 4°C, case 1a). At the same time, the relation of
\(N_F/N\) passes more sharply through its maximum, and at a temperature such as 53° C. the number of objects on the counting chamber is no greater than the initial number. This is due to the preliminary storage having increased the haemolysis of fragments at any given temperature. That this is probably the result of an effect on red cell shape is suggested by what is seen when the heating chamber (Ponder, 1949) is used to compare a system containing fresh cells with a system containing stored cells. In the latter, instead of defects originating centrally or peripherally and extending to split the cell into fragments of approximately equal size, small irregularities at the edge of the cell round up to form a number of minute fragments surrounding the remainder of the cell, which at the same time becomes spherical. As the temperature is increased, this sphere haemolyses without further fragmentation. The effect of storage is thus essentially an effect on red cell shape, a process involving sphere formation and haemolysis taking the place, to some extent, of the fragmentation process seen in the case of the washed discoidal red cells of fresh blood.

(iii) **Human red cells, washed and treated with lecithin.** These systems are prepared by washing human red cells with saline and then adding a sufficient quantity of a sol of distearyl lecithin (1 mg./ml. in saline) to bring the volume concentration to 0.4. After standing for 15–30 min. at room temperature, the cells appear between plastic surfaces as spheres or finely crenated spheres.

When heated, systems treated with distearyl lecithin show partial haemolysis (case 6a), the ghosts being haemoglobinized to different extents. The volume of the ghost is less than that of the intact cell, but all the ghosts remaining are clearly visible objects on the counting chamber; the value of \(F\) is accordingly calculated as \(N_F/N\). As the temperature is increased, \(N_F/N\) and \(F\) remain virtually constant, i.e. there is no fragmentation, but \(P\) increases (Fig. 3, man, lecithin, case 6a). This is a very clear case of haemolysis taking the place of fragmentation when the shape of the cell is changed from that of a disk to that of a sphere.

(iv) **Fresh human red cells in plasma.** The effect of heat on the cells of these systems differs greatly from that observed in systems containing washed red cells (Fig. 3, lower figure). Both fragmentation and haemolysis are less conspicuous when plasma is present, as may be seen by comparing the curves marked \(F\), saline with the curve marked \(F\), plasma, and the curve marked \(P\), saline with the curve marked \(P\), plasma. The relation between fragmentation and temperature is not linear in systems containing plasma, at least when \(F\) is calculated on the basis of the haemolysis being all-or-none. The situation will probably require much further investigation before it is clarified, but the presence or absence of plasma apparently has about the same effect on both fragmentation and haemolysis as a 4–5° C. difference in temperature (the value of \(F\) at 51° C. in the absence of plasma is roughly equal to the value of \(F\) at 56° C. in the presence of plasma, and the value of \(P\) at 52° C. in the absence of plasma is roughly the same as the value of \(P\) at 56° C. in the presence of plasma). It can also be shown that the remarkable inhibitory or protective effect of plasma on fragmentation haemolysis is largely due to the contained serum albumin, and it will be recalled that serum albumin is an anti-sphering substance responsible for the maintenance of the discoidal form of the red cell in many systems.
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These results may be clearer if considered in terms of a simple hypothesis. Fragmentation by heat is apparently an event which takes place during a transition of the red cell from a body with some degree of elasticity to a plastic body, and finally to a body which behaves as if it were a highly viscous fluid. If the cell is spherical, there is virtually no tendency to fragmentation, for the sphere itself is a very stable form, and the more nearly the cell approaches to a sphere, the more stable it is. If the cell is a disk, on the other hand, it may become unstable at some stage of the transition from its initial state to the final state of a viscous fluid. In the case of a cylinder of highly viscous material, instability usually occurs when the length of the cylinder is $2\pi r$, $r$ being the radius of the cylinder. Ignoring the material which fills the biconcavity and which becomes attenuated when the cell is about to fragment, the discoidal red cells can be regarded as the body generated by a circle of radius $r$ moving in a circle of radius $R$, $(R + r)$ being the semi-diameter of the cell and $r$ being half the thickness of the rim. Since $2\pi R > 2\pi r$, a viscous body of this shape will become unstable for the same reasons as a cylinder of the same material becomes unstable, and will tend to break into beads or fragments. The temperature $T_{0,F}$ at which this occurs, however, will be that at which the properties of the materials of which the cell is composed change from those of an elastic or plastic solid to those of a highly viscous fluid, and so $T_{0,F}$ will be determined by the particular architecture of the red cell ultrastructure. It is easy to understand that the presence of plasma can change $T_{0,F}$ substantially, for the presence or absence of plasma components has a very substantial effect on the surface ultrastructure at least, as shown by their effect on red cell shape.

(4) Fragmentation of the red cells of other mammals by heat

The conclusions that heating may result in the production of ghosts with differing properties, and that conditions which tend to render the cells spherical prevent its fragmentation, can be further illustrated by examining the fragmentation and haemolysis of the red cells of mammals other than man. Speaking somewhat generally, the value of $K_F$ is determined by the shape of the cells, for $K_F$ is zero when the cells are spherical and larger when the cells are flat disks at the temperature at which fragmentation takes place; the value of $T_{0,F}$, on the other hand, is determined by properties which have no necessary relation to shape, but which determine when the cell begins to behave as a viscous fluid instead of as an elastic or plastic solid. The properties of the ghosts, e.g. whether they possess rigidity or not, are again likely to be related to the architecture of the ultrastructure of the type of cell under consideration; the study of heat fragmentation of the red cells of different mammals may accordingly be expected to reveal a situation which is partly simple and partly complex.

Table 1 shows representative values for $K_F$ and for $T_{0,F}$ found for the red cells of man, rabbit, dog, cat, ox and sheep, this order being that of decreasing red cell diameter and volume. The relatively flat red cells of man, the rabbit, the dog, and the cat remain flat up to temperatures at which fragmentation occurs and give values of $K_F$ which range from 1.22 to 0.25; the values do not altogether correspond to the
red cell diameters or volumes, but $K_F$ is a constant which is easily affected by minor variations in the state of the cells, e.g. on the length of time after withdrawal from the animal (cf. Fig. 3), and so the correspondence is probably as good as one would expect. The same tendency for these relatively large and flat cells to undergo

<table>
<thead>
<tr>
<th>Animal</th>
<th>$K_F$</th>
<th>$T_{50}$</th>
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<tbody>
<tr>
<td>Man</td>
<td>1.22</td>
<td>49.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.50</td>
<td>52.6</td>
</tr>
<tr>
<td>Dog</td>
<td>0.25</td>
<td>53.0</td>
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<tr>
<td>Cat</td>
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<td>51.5</td>
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<tr>
<td>Sheep</td>
<td>0.02</td>
<td>55.5</td>
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Fig. 3. The variation of $F$ (dots), $P$ (circles), and $N_F/N$ (dotted line) with temperature $T$ (abscissa). Upper left, fresh human red cells, freshly washed; upper middle, human red cells, kept at 4°C for 48 hr., then washed; upper right, human red cells, washed, and treated with lecithin. Lower, fresh human red cells in saline and in plasma. For description, see text.

fragmentation is seen when they are observed directly on the heating chamber; one is struck by the fact that any crenations which are present initially tend to disappear as temperatures in the neighbourhood of 45°C are reached, and that the cells of the rabbit, dog, and cat undergo fragmentation at higher temperatures in much the same way as human red cells do, although the number and size of fragment per cell is smaller. The red cells of the ox and sheep, on the other hand, are less flat to begin
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with; they become spherical at temperatures in the neighbourhood of 50° C., and at higher temperatures they haemolyse almost without fragmentation. It is an interesting and unforeseen result that \( T_{0,F} \) increases as \( K_F \) decreases; this presumably means that both constants measure related attributes of the architecture of the red cell ultrastructure.

Heat haemolysis of the red cells of man and of the dog is substantially all-or-none; haemolysis of the red cells of the rabbit, the cat, and the sheep, on the other hand, is an example of a process which is described either by case 5a or 6a, i.e. the ghosts have rigidity, complete or partial, and are partially haemolysed. The reason for these differences in the properties of the ghost remains to be investigated.

SUMMARY

A method is described by means of which the fragmentation and haemolysis resulting from heat can be studied quantitatively, and by means of which certain situations as regards the rigidity and the extent of haemoglobinization of the ghost can be distinguished from each other.

Fragmentation and haemolysis increase with time so as to become virtually complete after 15 min. heating at any temperature. The fragmentation and haemolysis at the end of 15 min. is linear with the temperature, and so can be expressed in terms of four constants, two referring to the former process and two to the latter.

Heating of human red cells under different conditions may give rise to ghosts with different properties as regards rigidity and degree of haemoglobinization. Conditions which render the cells spherical prevent its fragmentation, and plasma contains substances (albumins) which inhibit both fragmentation and haemolysis by heat. Similar conclusions can be reached by examining the heat fragmentation and haemolysis of the red cells of mammals other than man.

REFERENCES