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In summary, human red cells processed in a closed system Cohn centrifuge and stored in glycerol at -80° C and -120° C have been transfused into recipients after preservation up to 28 months. The sterility of the system made possible additional post-thawing storage of the cell at 4° C for distribution to the hospital where the transfusions were administered. No transfusion reactions of any kind were noted. The cells appeared completely similar to standard ACD cells stored up to 21 days by standard methods.

Radioactive Tracer Studies of Red Cell Survival in Tumour-Bearing Rats

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Anaemia associated with a reduction in life span of the circulating red cells is a frequent finding in cases of human cancer (Price and Greenfield, 1958). The question to what extent the observed effects are due to intrinsic red cell defects or to immune or humoral factors causing increased red cell destruction has yet to be answered conclusively. Studies of the auto-survival of 14C-labelled red cells in leukaemic patients (Berlin et al., 1954) suggest an intrinsic defect in the patients' own red cells. On the other hand, Miller et al. (1956) demonstrated by differential agglutination and 51Cr-labelling techniques a reduced life span both of red cells auto-transfused to cancer patients, and of red cells transfused from normal subjects to cancer patients. Hyman et al. (1956) also found a shortened life span of 51Cr-labelled cells transfused from normal subjects to cancer patients, whilst cells transfused from cancer patients to normal volunteers had a normal or nearly normal survival. These results would appear to favour the hypothesis of an extra-corpuscular destruction mechanism. Greenfield and Price (1958) in studies with 51Cr- and 59Fe-labelled cells in animals bearing tumours found deposits of the isotopic label within haemorrhagic or necrotic regions of the tumour; they suggested that vascular lesions in the tumour bed may play an important role in red cell destruction in cancer. The work of Ponder (1945), Gross (1949), Adelsberger (1951), Baldwin (1957), Green (1957) and their co-workers has shown that toxins, lysins or antibodies present in or produced by tumour tissue may also be active in causing increased red cell destruction.

In the work described below, red cell destruction has been studied in rats of the GÇôAugustGÇ¥ R2426 strain bearing a transplantable mammary adenocarcinoma. Red
cell life span has been measured by taking blood from donor animals, labelling the red cells in vitro with 51Cr, and injecting 0.5 ml of the labelled red cell suspension into litter mate recipients; 0.02 ml blood samples are then withdrawn daily from the cut tails of the recipients for assay of 51Cr content. The experimental data are corrected for the increase in blood volume with growth during the period of study. The corrected values of 51Cr concentration in blood are plotted semi-logarithmically against time; in normal rats, the 51Cr half-clearance time is found to be 20.7 ± 2.5 days (Belcher and Harriss, 1957).

Preliminary results of these studies have already been reported (Belcher, 1957). Animals receiving subcutaneous implants of the tumour studied are observed to undergo an acute haemolytic episode 10-15 days after implantation. This episode may be fatal, but if the animal survives, the haemoglobin concentration returns to normal within a further 10 days. The episode may be attributed to agglutinating anti-bodies evoked in response to the tumour graft, though confirmatory evidence from serological studies of the existence of an immune mechanism has yet to be obtained.

Fig. 1. Blood haemoglobin and 61Cr concentrations in two GÇ£AugustGÇ¥ rats transfused with 61Cr-labelled cells from normal litter mate donor 4 days before receiving tumour implant. Normal 61Cr survival shaded.

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By transfusing 51Cr-labelled cells to the host animals before implantation of tumour, or after implantation but before the start of the episode, the fate of the transfused cells may be followed during the haemolytic episode (fig. 1 ). Up to the start of the episode, survival is normal, but during the episode almost all the labelled cells are removed in an exponential fashion, the 51Cr concentration falling to less than 10 % of its initial value.

If labelled cells are transfused from normal donors to animals which have already suffered such a haemolytic episode and are in recovery from it, red cell destruction is found to be only slightly more rapid than normal. It thus appears

Fig. 2

Fig. 3

Fig. 2. Below: Blood haemoglobin and 51Cr concentration in two normal GÇ£AugustGÇ¥ rats transfused with 61Cr-labelled red cells from tumour-bearing litter mate donor 11 days after tumour
that during the recovery phase, either the concentration of the haemolytic agent or
the sensitivity of the red cells to the agent is reduced in some manner.
If 51Cr-labelled red cells are transfused from a tumour-bearing donor to normal
recipients, the recipient animals undergo a delayed haemolytic episode similar to
that observed in normal animals receiving tumour implants. Similar effects are
observed whether the labelled cells are injected intravenously or intraperitoneally;
in the latter situation the labelled cells appear quantitatively in the circulating
blood within a few hours. The delay in onset of the episode and the fate of the
labelled cells vary according to the time after implantation at which blood is taken
from the donor animal. If transfusion is performed shortly before or during the
development of the haemolytic episode in the donor, the episode in the recipient is
almost immediate, and affects both the transfused labelled cells and the recipientGÇÖs
own cells (fig. 2). If, however, blood is transfused from animals in recovery from
their haemolytic episode, the episode in the recipient is delayed some days, and
involves only the recipientGÇÖs own cells, the transfused labelled cells disappearing at a
steady rate which is only slightly more rapid than normal (fig. 3). Account must be
taken of the abnormal age-distribution of the labelled cells in interpreting the latter
results, but it is clear that the 51Cr survival curve shows no discontinuity comparable
with the fall in haemoglobin concentration which marks the onset of haemolysis.
It thus appears that the donorGÇÖs red cells which have become insensitive to the action
of the haemolytic agent retain this desensitisation when transfused to a normal
recipient, despite the fact that they carry the agent.
The above results show that, at least in rats bearing the type of tumour studied,
haemolytic processes, probably immune in nature, are of great importance in determining
the life-span of the host animalGÇÖs red cells or of transfused cells. Further
studies designed to establish the nature of the observed effects and their importance
in other species and other types of tumour are in progress.

References

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