

Extrusion of Nuclei of Transfused Avian Erythrocytes in the Mammalian Spleen

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THERE IS convincing evidence from clinical studies that in human disease the spleen may selectively retain abnormal red cells and that their destruction is thereby accelerated.¹ The mechanisms concerned are by no means clear, but may include varying degrees of phagocytosis, formation of auto-antibodies, liberation of tissue lysins such as lysophosphatides,³ as well as intracellular disturbances of metabolism as a result of stagnation.² Whether indeed the spleen exerts any unique destructive action apart from its being peculiarly and conspicuously a site for erythrosthesis appears uncertain.

To examine experimentally the matter of what happens to red cells in the spleen it was decided to transfuse into rodents the red cells of ducks, which are readily distinguished histologically by the nuclei, and to follow events in the spleen and other viscera over a period of time by sacrificing the recipient animals at intervals. The results include an observation not hitherto reported: the apparent extrusion of the nucleus by duck cells lying free in the spleens of guinea-pigs. The finding may be of interest as an illustration of one type of dynamic change in the nucleated erythrocyte. Moreover, experience thus far indicates that it may well be somehow specifically related to the spleen.

METHODS

Duck blood cells were washed three times with 0.9 per cent saline and finally suspended in an equal volume of saline. Of this, 0.2 to 1.0 cc. were injected intravenously into mice, 2.0 to 4.0 cc. into guinea pigs, and 5.0 to 10.0 cc. into rabbits. The animals were sacrificed at intervals running from 15 minutes to 36 hours after injection of duck cells. Imprints were made on glass slides from the cut surfaces of lungs, liver, and spleen, and from peripheral blood and bone marrow. These were stained with Wright's stain. The blood serum was examined for hemolysis and the bladder urine for hemoglobin.

Rabbits were immunized by daily intravenous injections of duck cells for 4 days and the serum collected 10 days after the last injection. This rabbit anti-duck serum was used to sensitize duck cells for certain *in vivo* and *in vitro* experiments.

For convenience, duck cells were sometimes used after a few days' storage at 4 C. in standard acid-citrate-dextrose solution. No significant difference was seen between the behavior of these cells and fresh, unpreserved cells.

RESULTS

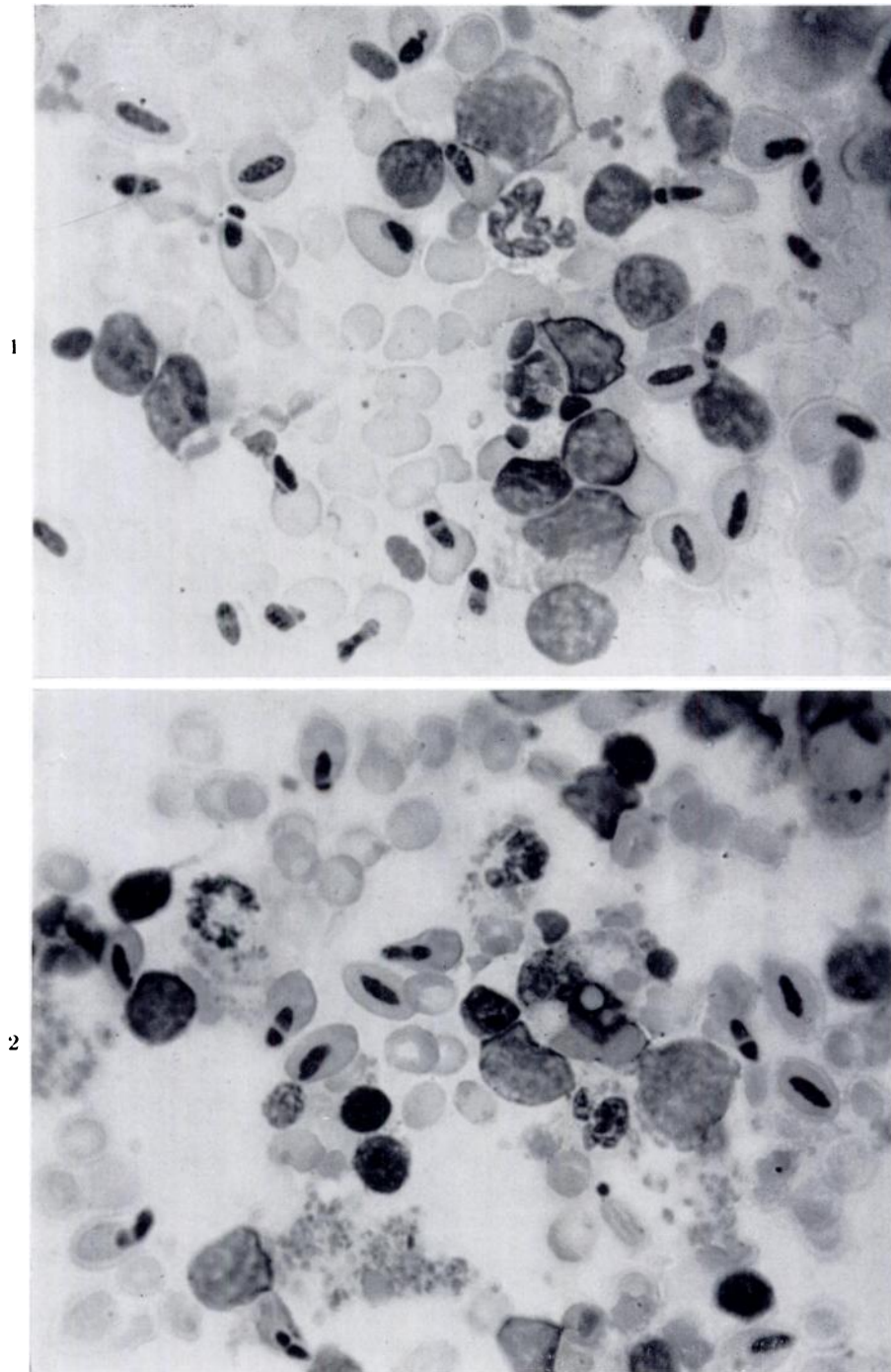
As might be expected, the findings varied not only with time and with the species of the recipient, but also somewhat from organ to organ in the same animal.

In the rabbit, destruction of duck red cells was rapid and apparently resulted largely from intravascular hemolysis. Within 15 to 30 minutes hemoglobinuria and hemoglobinemia were regularly observed, and microscopic examination of the organs showed only rare duck cells as survivors.

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FIGURES 1 and 2

On the other hand, in the mouse appreciable hemolysis did not occur, at least within six hours after injection of duck cells. Agglutination was a regular feature, particularly in the spleen and bone marrow where phagocytosis was also observed.

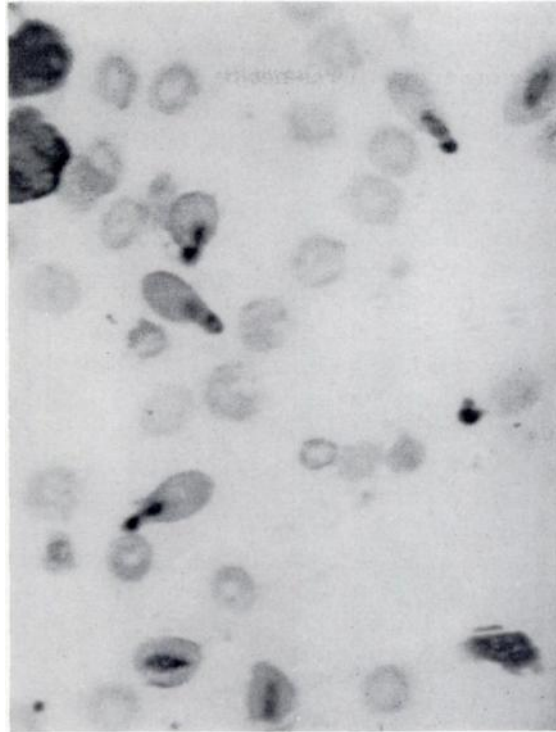


FIGURE 3

In the guinea pig, destruction of duck erythrocytes took place more slowly than in the rabbit, for hemolysis was inconspicuous. However, there appeared to be rather more phagocytosis and hemagglutination than in mouse tissues.

Of more interest was the observation illustrated in the accompanying photographs. This consists in the apparent extrusion of the nucleus by duck cells lying free as seen in imprints of guinea pig spleens. Stages of this process could be identified from (1) the early movement of the nucleus to one end of the elliptical cell, through (2) the emergence of part of the nucleus outside of the erythrocyte wall, until (3) the nucleus was completely ejected. There did not appear to be accompanying lysis, or, indeed, any appreciable loss of hemoglobin. Often the nuclei gave the appearance of some segmentation but this was probably due to constriction by the cytoplasmic membrane rather than to amitotic division.

In wet preparations, using the ordinary microscope, distortions of the form of duck cells due to the emerging nucleus could readily be made out. It did not appear that nuclear extrusion was rapid: single cells observed for 30 to 60 minutes showed no change. However, the technical conditions were not satisfactory enough to draw any conclusions; the use of phase-contrast microscopy with temperature control might disclose a more detailed and acceptable view of the process.

The phenomenon has been observed regularly in guinea pigs sacrificed between three and five hours after injection of duck cells. It was encountered only irregularly in animals killed within an hour after injection, and in those examined after 18 hours duck cells could no longer be identified. In all of 17 guinea

pigs sacrificed after the appropriate interval, 10 to 25 per cent of the duck red cells in the spleens exhibited some stage of nuclear extrusion.

The process of nuclear extrusion has not been seen except in the guinea pig spleen. In about half of the animals, what were taken to be extruded nuclei of duck erythrocytes were seen in imprints of the liver. But it is difficult to be certain of the origin of nuclear material entirely free of cytoplasm, even though in this case the distinctive size and shape of the avian red cell nucleus is helpful. Moreover, even if it be admitted that these were in fact duck nuclei, they might have come from the spleen via the portal system, since actual nuclear extrusion was not observed in the liver. In the lung and bone marrow less than 5 per cent of the duck cells showed some eccentricity in the position of the nucleus. Again, the significance of this is open to question and it can only be said that nuclear extrusion like that in the spleen was not seen.

The injection into guinea pigs of duck cells suspended in a rabbit anti-duck-cell antiserum resulted only in accelerated hemolysis, with rapid production of hemoglobinemia and hemoglobinuria. Duck cells were not identifiable in the tissues.

Mice were injected intravenously with duck cells suspended in normal guinea pig serum rather than saline, but while hemolysis did not appear to be accelerated, neither was there evidence of nuclear extrusion.

Duck cells have been injected intraperitoneally into guinea pigs, yet after intervals up to 24 hours most of those recovered appeared intact microscopically and no significant change in the nucleus was seen.

Attempts to reproduce nuclear extrusion *in vitro*, with experimental conditions involving stagnation, anoxia, hemolytic antibodies, spleen, and splenic extracts have so far failed. Duck cells allowed to stand at 37 C. in guinea pig serum for several hours exhibited minor degrees of agglutination and slight hemolysis, but there were no morphologic changes in the unlysed cells. Similar preparations equilibrated with N₂ or CO₂ gave identical results.

Minces of guinea pig spleen were mixed with 0.1 cc. of duck cell suspension and observed as long as 16 hours, but no change in the duck erythrocytes was noted. Negative results were also obtained with lyophilized ox spleen.

DISCUSSION

The findings to date suggest that at least two factors may be related to the observed nuclear transformations: (a) the animal species, (b) the intact spleen. As for the first, it may well be that species differences reflect the operation of humoral substances, e.g., antibody. It could be that in the guinea pig the plasma constituents are quantitatively or qualitatively appropriately unlike those of the mouse. If this were so, perhaps variation of the species of avian donor would also influence the findings, but studies of this kind have not yet been made. With regard to the apparent essentiality of an intact spleen, it can only be said that nuclear extrusion has been seen with great regularity in that organ and not elsewhere. Further experience may show that this is a quantitative, rather than a qualitative difference. On the other hand, the failure to reproduce nuclear extrusion *in vitro* suggests that the spleen has functioned as something more than a site for erythrocytosis. Moreover, from the large size of the duck erythrocytes one would expect them to be unable to pass readily through the capillaries of many organs of the guinea pig.

It is worth noting that nuclear extrusion is not described in the various investigations by others of hemolysis of avian erythrocytes. Lysis *in vitro* is associated rather with loss of blood pigment, leaving oval ghosts with their nuclei.⁴

On the other hand, the natural formation of erythroplastids in salamanders seems to result from cytoplasmic segmentation and a separation of the nucleus^{5, 6} which may be to some extent analogous to the changes described above for duck erythrocytes. It would be interesting to know whether erythroplastid formation takes place in any exceptional manner in the salamander spleen. Nothing of the sort has been seen in duck spleen.

The number of observations must be extended before any conclusions can be drawn about the significance of the finding now reported, in respect of both (a) its relation to the spleen, and (b) its bearing on the general problem of loss of the nucleus of the erythrocyte. Nonetheless, on the basis of available data the possibility exists that the extrusion of nuclei from duck erythrocytes as observed in guinea pig spleens may be specifically related to some singular property of splenic tissue.

SUMMARY

1. Duck erythrocytes have been injected intravenously into rabbits, mice, and guinea pigs and at intervals thereafter microscopic examination of certain organs has been made.

2. In the guinea pig spleen extrusion of the nuclei of the duck cells has been observed with great regularity.

3. Attempts to reproduce this phenomenon *in vitro* have failed.

4. It is suggested that nuclear extrusion of this kind is conditioned by (a) the species into which the cells are transfused and (b) an intact spleen.

SUMMARIO IN INTERLINGUA

1. Erythrocytos de anate esseva injicite intravenosemente in conilios, muses, e porcicos de India. Postea intervallate examines microscopic de certe organos esseva executate.

2. In le splen de porcicos de India, extrusion del nucleos del erythrocytos de anate esseva observate con alte grados de regularitate.

3. Essayos de reproducere le phenomeno *in vitro* non succedeva.

4. Es proponite que le extrusion nuclear de iste genere es conditionate per (a) le specie del recipiente del transfusion de erythrocytos e (b) le splen in stato intacte.

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