# Technical Papers

## Comparison of *in Vitro* and *in Vivo* Radioiron Uptake by Pigeon Erythrocytes

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Results from recent studies on the uptake of radioactive iron by avian erythrocytes and mammalian reticulocytes *in vitro* have been described by several investigators (*1–4*). It seems to be generally agreed that the iron taken up by nucleated or immature red cells *in vitro* is utilized for heme synthesis in a manner analogous to that of the corresponding iron *in vivo*. The distribution of the iron taken up in pigeon red cells and a discussion of some points of difference between the results from *in vitro* and *in vivo* experiments are the contents of this report (*5*).

$Fe^{59}$ was added to pigeon whole blood in amounts of 1.5 μg $Fe^{59}$ (0.08 μc) to 5 ml of blood. Samples were then maintained in a water bath at 37°C for various periods of time. Nuclei and stroma were separated from cytoplasm by Parpart's method (*6*). The nuclei and stroma were washed six times, and the washings were added to the cytoplasm that had been obtained by hemolysis. Nonhemin iron was extracted from the nuclei and stroma by using the hot-pyrophosphate method described by Brueckmann and Zondeck (*7*). Radioactivity was determined by means of a well-type scintillation counter.

Table 1 gives the data for pigeon whole blood incubated with $Fe^{59}$ for various time intervals. Notice that after an incubation period of only 15 min an appreciable amount of radioactive iron is already taken up by pigeon erythrocytes and that this amount tends to increase as the incubation time increases. Most of the iron present in the cytoplasm is presumed to be hemoglobin iron and it, too, increases gradually as incubation continues. On the other hand, the percentage of iron taken up by the nuclei and stroma is relatively high at the early stage of incubation but shows a tendency to decrease during the incubation period.

The nature of the iron present in nuclei and stroma remains obscure, although several investigators (*8, 9*) have suggested explanations. Other investigators (*3, 10, 11*) have observed the presence of nonhemin iron in avian red cells, but none has decided its exact localization in the cells. Thus, a nonhemin iron extraction of the nuclei and stroma was performed in order to determine the nature of the iron in this fraction. The data in the last two columns of Table 1 show that the percentage of nonhemin iron in the nuclei and stroma is very high, particularly at the early stage of incubation. Thereafter, there is a decline. To explain the presence of radioactive hemin iron in the nuclei and stroma, we concur with the possibilities suggested by Tishkoff *et al.* (*8*) and Moskowitz *et al.* (*9*); that is, that there is contamination by hemoglobin and incorporation into some other hemin substances contained in nuclei and stroma. However, we do not believe that the problem is completely solved.

The experiments next described were designed to compare iron uptake by pigeon red cells *in vitro* and *in vivo*. Various amounts of radioactive iron were given intravenously to pigeons, and blood samples were taken by cardiac puncture at appropriate time intervals after injection. Whole blood samples were analyzed for radioactive iron content in the aforedescribed manner. The results of two such experiments are given in Table 2.

The percentage of iron taken up per milliliter of packed red cells *in vivo* was much lower during the time measured than that in the *in vitro* experiments. Although there are several explanations for this observation, the most likely one is that the iron is taken up not only by peripheral red cells but primarily by erythroid precursors in the bone marrow. Conse-

Table 1. *In vitro* uptake of $Fe^{59}$ (expressed as percentages) by pigeon erythrocytes at various time intervals and its approximate intercellular distribution.

| Time of incubation (hr) | Uptake of $Fe^{59}$ per ml of P.R.C. | $Fe^{59}$ per ml of P.R.C. | | $Fe^{59}$ in nuclei and stroma per ml of P.R.C. | |
|---|---|---|---|---|---|
| | | Cytoplasm | Nuclei and stroma | Nonhemin | Hemin* |
| Expt. 1 | | | | | |
| ¼ | 1.69 | 43.2 | 59.6 | 43.0 | 16.6 |
| ½ | 3.78 | 70.8 | 42.4 | 31.2 | 11.2 |
| 1 | 4.84 | 74.2 | 25.2 | 14.6 | 10.6 |
| 6 | 8.30 | 84.0 | 11.0 | 6.3 | 4.7 |
| Expt. 2 | | | | | |
| 1 | 4.06 | 60.2 | 41.2 | 31.0 | 10.2 |
| 3 | 7.18 | 71.3 | 28.2 | 21.0 | 7.2 |

*Hemin $Fe^{59}$ obtained by the subtraction of nonhemin $Fe^{59}$ from percentage of $Fe^{59}$ in nuclei and stroma.

Table 2. *In vivo* $Fe^{59}$ uptake (in percentages) by pigeon erythrocytes and its intercellular distribution at various time intervals after intravenous injection.

| Time after injection (hr) | Uptake of $Fe^{59}$ per ml of P.R.C. | $Fe^{59}$ per ml of P.R.C. | | $Fe^{59}$ in nuclei and stroma per ml of P.R.C. | |
|---|---|---|---|---|---|
| | | Cytoplasm | Nuclei and stroma | Nonhemin | Hemin |
| Expt. 3 | | (Pigeon weighing 320 g was given 22 μg (1.2 μc) of $Fe^{59}$) | | | |
| ½ | 1.18 | 92.5 | 8.12 | 1.12 | 7.00 |
| 1½ | 1.64 | 93.4 | 7.70 | 1.24 | 6.46 |
| 2 | 1.66 | 97.0 | 7.60 | 1.11 | 6.49 |
| 3 | 1.88 | 93.5 | 8.00 | 2.11 | 5.89 |
| Expt. 4 | | (Pigeon weighing 359 g was given 18 μg (0.96 μc) of $Fe^{59}$) | | | |
| ½ | 0.62 | 92.5 | 7.42 | 3.80 | 3.62 |
| 2 | 1.35 | 92.0 | 8.07 | 4.06 | 4.01 |
| 3 | 1.81 | 93.5 | 6.19 | 2.65 | 3.54 |
| 4 | 2.20 | 94.0 | 6.00 | 1.90 | 4.10 |

quently, the length of time of the experiments was too short to permit the appearance of large amounts of $Fe^{59}$-labeled hemoglobin in peripheral red cells. As was expected, the data show a low uptake of iron by the peripheral erythrocytes during the time this was being measured.

A comparison of the data in Tables 1 and 3 reveals that there is a noteworthy difference in the mechanism of $Fe^{59}$ uptake by pigeon erythrocytes *in vitro* and *in vivo,* especially in the early stages of incubation. During the time of incubation, the amount of iron taken into nuclei and into stroma in *in vivo* experiments was remarkably lower, and the percentage of $Fe^{59}$ in the cytoplasm was considerably higher, than that seen *in vitro.*

The experiments covered by Table 3 were designed to determine whether there is a difference in the nature of the nonhemin iron present in nuclei and stroma after *in vitro* and *in vivo* uptake. Pigeon whole blood was incubated with $Fe^{59}$ for 15 min, washed three times with iron-free isotonic saline, and then resuspended to its original concentration in iron-free isotonic saline (sec. A), in isotonic saline containing 107 μg percent of stable iron (sec. B), or in pigeon plasma (serum iron 105 μg percent) (sec. C). The samples were then reincubated, respectively, for 1 or 3 hr in a water bath at 37°C. For comparison, a pigeon weighing 260 g was given 12 μg (0.64 μc) of $Fe^{59}$ intravenously and was bled after 18 hr. The red cells were separated from the plasma, washed three times, and then incubated in iron-free isotonic saline (sec. D).

Table 3. Comparative alterations in $Fe^{59}$ (in percentages) taken up both *in vitro* and *in vivo* by pigeon erythrocytes after washing with mediums containing various amounts of iron.

| Time of reincubation (hr) | $Fe^{59}$ per ml of P.R.C. | $Fe^{59}$ per ml of P.R.C. | | $Fe^{59}$ in nuclei and stroma per ml of P.R.C. | |
|---|---|---|---|---|---|
| | | Cytoplasm | Nuclei and stroma | Nonhemin | Hemin |
| Section A | | | | | |
| Before reincubation | 100.0 | 37.1 | 64.8 | 56.4 | 8.4 |
| 1 | 85.6 | 42.0 | 50.0 | 41.5 | 8.5 |
| 3 | 57.0 | 45.2 | 20.0 | 15.1 | 4.9 |
| Section B | | | | | |
| Before reincubation | 100.0 | 25.4 | 72.8 | 59.8 | 13.0 |
| 1 | 85.3 | 33.5 | 57.0 | 39.3 | 18.7 |
| 3 | 79.8 | 35.3 | 49.4 | 38.5 | 10.9 |
| Section C | | | | | |
| Before reincubation | 100.0 | 25.4 | 72.8 | 59.8 | 13.0 |
| 1 | 80.5 | 32.7 | 53.6 | 47.5 | 5.1 |
| 3 | 72.4 | 36.5 | 44.2 | 30.1 | 14.1 |
| Section D | | | | | |
| Before reincubation | 100.0 | 81.0 | 20.4 | 12.1 | 8.3 |
| 1 | 97.2 | 81.2 | 17.8 | 10.0 | 7.8 |
| 3 | 91.2 | 81.2 | 16.8 | 8.0 | 8.6 |

The data show that the radioactive iron, which is taken up by pigeon erythrocytes *in vitro,* leaves the cells during reincubation in the various mediums described. This is particularly evident in the iron-free medium. However, the percentage of $Fe^{59}$ present in cytoplasm increased during reincubation, whereas the percentage of $Fe^{59}$ present in the nuclei and stroma declined, primarily because of a considerable drop in the nonhemin iron fraction. This strongly suggests that the nonhemin iron in nuclei and stroma can be utilized for hemoglobin synthesis and that it may be in equilibrium with the iron in the suspending medium. In contrast to these observations, the iron, which is taken up by pigeon red cells *in vivo,* is comparatively stable and undergoes only a very slight decline, which is attributable to the nonhemin iron fraction of nuclei and stroma.

This fact suggests the possibility that the iron taken up by pigeon erythrocytes *in vitro* at the early stage of incubation is attached or combined loosely to the surface of the cells and may be in equilibrium with iron present within them, particularly in the nuclei and stroma.

**References and Notes**


* Rockefeller Foundation fellow from the University of Tokyo.

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23 March 1954.


## New Method of Intracoelomic Grafting


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The technique of intracoelomic grafting, originally described by Hamburger (*1, 2*), has found wide application in the field of experimental embryology, and the results obtained through its use have contributed much to our understanding of developmental processes. The technique in its present stage of development (*3*) has, however, certain inherent limitations that restrict its usefulness. One drawback is that the size of the implant is limited both by the small capacity of the coelom of 60- to 70-hr-old chicks and by the necessarily small incision permitted in gaining access to it. For these reasons, successful implantation of relatively large bits of tissue—for example, approximately 0.5 to 1.0 $mm^3$—is not practicable.

During a recent investigation, it became necessary to make intracoelomic grafts of the thyroid glands of 10- to 11-day-old chick embryos. It was soon discovered that implantation of a half or even a quarter of such a gland was difficult, if not impossible, and that the results to be expected from such a procedure were highly questionable. To circumvent this impasse, a new approach was required. The method that was finally developed appears to have sufficient applicability to be of interest to workers in fields other than embryology.

By 3½ days of development (*4,* Hamburger-Hamilton stage 20), the chick embryo lies entirely on its left side, the allantois is beginning to expand, and the embryonic membranes have grown completely over the embryo and have separated into chorion and amnion. The embryonic coelom and the extraembryonic coelom are still in broad communication at this time, for the umbilical ring has not yet been occluded by the structures that pass through it. Beginning with the time that the embryo first comes to lie completely on its side and ending with the time that the allantois has expanded to such a point that the site of operation is obstructed (approximately Hamburger-Hamilton stage 23), the coelom of the chick is readily accessible for operative manipulations and is in a condition such that tissue placed within its confines readily becomes vascularized.

The operation itself is extremely easy and rapid. The egg is opened in the usual manner, and the embryo is lowered by withdrawing a small amount of albumen from the pointed end of the egg. The opening required in the chorion (Fig. 1 *A* and *B*) may be made either by cutting with iridectomy scissors or by tearing with fine watchmaker's forceps, and should be just large enough to admit the graft; it is essential to distinguish carefully the chorion and amnion and to avoid injury to the latter. The graft is temporarily placed on the chorion next to the incision, and, to facilitate its placement and later recovery, it is marked with a few grains of sterile blood carbon or, alternatively, with a vital dye. Using an L-shaped blunt glass instrument (Fig. 1), the graft is nudged into the incision and gently pushed past the anterior face of the allantois through the umbilical ring. Upon reaching the dorsal surface of the embryonic coelomic cavity (Fig. 1 *B*), the graft may be directed toward the posterior portion of the cavity and pushed into a secure position. It is quite possible to push the graft into the anterior portion of the coelomic cavity at this point. The carbon particles are usually visible through the flank of the embryo and will indicate by their location whether or not the graft has become firmly lodged in the desired location. The volume of tissue grafted is largely dependent upon its shape. A spherical or cubical graft having a volume approaching that of a thin elongated graft is more difficult to implant than the latter, which may be inserted without unduly distorting the embryo. When the graft has been placed in position, the albumen is replaced, the shell window is fitted into place, and both openings are sealed with melted paraffin.