line and the presence of these cells in all the 3 groups has some evolutionary bearing.

These acidophilic cells in the optic lobes of the spider *Cryptophora* sp. constitute a new group of cells in spiders. At the moment, their function can only be speculated upon, but from their location it would appear that they may be somehow connected with the eyes and respond to changes in the light intensity or photoperiod.

1 We wish to express our thanks to Prof. U.S. Srivastava for providing working facilities and valuable suggestions.

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PRO EXPERIMENTIS

Relevance of specific activity in experimental erythrocytocide by ^{55}Fe[1]

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Summary. Iron loads between 0.20 µg and 26 µg, added to 5 µCi ^{59}Fe, were followed for up to 150 days in mice. Relative organ uptake increased as a function of iron load in liver and kidneys while it decreased in bone marrow and blood. Several weeks after injection, all load-related differences disappeared.

The limited range of Auger electrons emitted by ^{55}Fe allows 94% of their energy to be absorbed[2] within a tissue sphere of 300 µm^3. Since X-rays contribute very little irradiation to adjacent tissue ^{55}Fe is a useful cytocidal agent[3,4]. Red blood cell formation, temporarily abolished by ^{55}Fe, recovers speedily due to increased manufacture of pronormoblasts[5]. The isotope has a 2.7 year half-life and eventually causes bone marrow aplasia, leukemia and osteosarcoma[6,7]. Our observations were made in mice after single i.v. injections of $^{55}FeCl_3$ of high specific activity ($\geqslant 1$ mCi/µg Fe). This ^{55}Fe was cyclotron produced to order and very costly. Reactor-produced ^{55}Fe contains only 10–40 µCi/µg Fe. Cyclotron produced ^{55}Fe will be designated as 'carrier-free' in commercial catalogues since no carrier iron is added deliberately. In view of the variable iron content of commercial ^{55}Fe it appears worthwhile to discuss the relevance of specific activity for experimental ^{55}Fe cytocide. Bone marrow radioiron uptake depends not only on the

^{59}Fe retention in percent of injected dose

Tissue	µg Fe	Day 1 x̄ SE	Day 2 x̄ SE	Day 24 x̄ SE	Day 63 x̄ SE	Day 150 x̄ SE
Blood*	0.2	39.5 ±12.3 (3)	42.7 ±10.6 (5)	51.8 ±3.2 (3)	23.6 ±1.8 (5)	9.2 ±- (1)
	1	46.4 ± 7.8 (3)	53.9 ± 8.4 (5)	41.5 ±7.8 (3)	20.4 ±2.2 (5)	11.9 ±- (2)
	3	40.3 ± 4.9 (3)	38.1 ±10.4 (5)	46.3 ±- (2)	20.7 ±1.2 (5)	11.0 ±2.9 (4)
	5	34.1 ±14.0 (3)	36.7 ± 9.9 (5)	43.3 ±4.9 (3)	22.8 ±1.5 (4)	13.3 ±1.5 (4)
	10	23.6 ± 6.2 (4)	19.7 ± 6.6 (4)	38.7 ±2.7 (3)	22.5 ±1.3 (5)	9.7 ±0.9 (3)
Liver	0.2	10.3 ± 1.9 (3)	11.9 ± 1.9 (5)	9.3 ±0.6 (3)	9.4 ±0.6 (5)	7.0 ±- (1)
	1	16.4 ± 2.1 (3)	11.6 ± 0.6 (5)	11.2 ±3.3 (3)	9.4 ±6.9 (5)	6.5 ±- (2)
	3	15.0 ± 3.1 (3)	19.9 ± 3.8 (5)	11.4 ±- (2)	10.9 ±1.1 (5)	5.7 ±0.5 (4)
	5	23.2 ± 3.7 (3)	21.8 ± 4.3 (4)	14.5 ±1.6 (3)	12.1 ±0.9 (4)	6.9 ±0.4 (4)
	10	26.2 ± 6.3 (3)	32.5 ± 2.6 (4)	19.4 ±1.9 (3)	12.9 ±1.5 (5)	5.2 ±0.3 (3)
Kidneys	0.2	1.6 ± 0.08	2.4 ± 0.46	1.3 ±0.10	0.64±0.04	0.44±-
	1	1.3 ± 0.18	1.4 ± 0.08	1.4 ±0.74	0.74±0.08	0.39±-
	3	2.4 ± 0.47	3.3 ± 0.69	1.4 ±-	0.85±0.08	0.52±0.04
	5	2.9 ± 0.89	2.4 ± 0.82	1.5 ±0.15	0.97±0.09	0.44±0.03
	10	2.1 ± 0.51	3.0 ± 0.49	1.5 ±0.25	0.81±0.04	0.46±0.09
Hind leg**	0.2	1.33± 0.17	0.74± 0.09	0.29±0.03	0.30±0.03	0.17±-
	1	0.84± 0.17	0.55± 0.03	0.29±0.03	0.27±0.02	0.14±-
	3	1.01± 0.28	0.55± 0.04	0.45±-	0.33±0.03	0.20±0.04
	5	0.94± 0.09	0.66± 0.13	0.53±0.12	0.50±0.02	0.14±0.01
	10	0.83± 0.16	0.65± 0.05	0.47±0.04	0.31±0.03	0.13±0.02
Heart	0.2	0.14± 0.01	0.27± 0.06	0.18±0.01	0.18±0.02	0.15±-
	1	0.12± 0.02	0.14± 0.01	0.15±0.03	0.24±0.04	0.15±-
3	3	0.17± 0.05	0.23± 0.05	0.22±-	0.34±0.04	0.18±0.02
	5	0.23± 0.06	0.22± 0.04	0.24±0.03	0.28±0.02	0.23±0.04
	10	0.17± 0.03	0.24± 0.02	0.22±0.02	0.28±0.02	0.17±0.00

*Total blood volume assumed to be 5% of b.wt.[18]. **Femur, tibia and fibula cleaned free of muscle. This constitutes about 5% of the bone marrow[8]. Fe uptake and retention in mice injected with 5 µCi and 0.2, 1, 3, 5, or 10 µg total iron. The second of 2 experiments is shown. Samples of 200 µl blood were obtained from ether-anesthetized mice prior to perfusion with physiological saline and organ removal, and whole blood values were computed assuming a blood volume of 5% b.wt. No spleens were taken because of their resistance to complete perfusion. Radioactivity of whole organs was counted in a well type scintillation counter. Number of mice used is indicated in parenthesis.

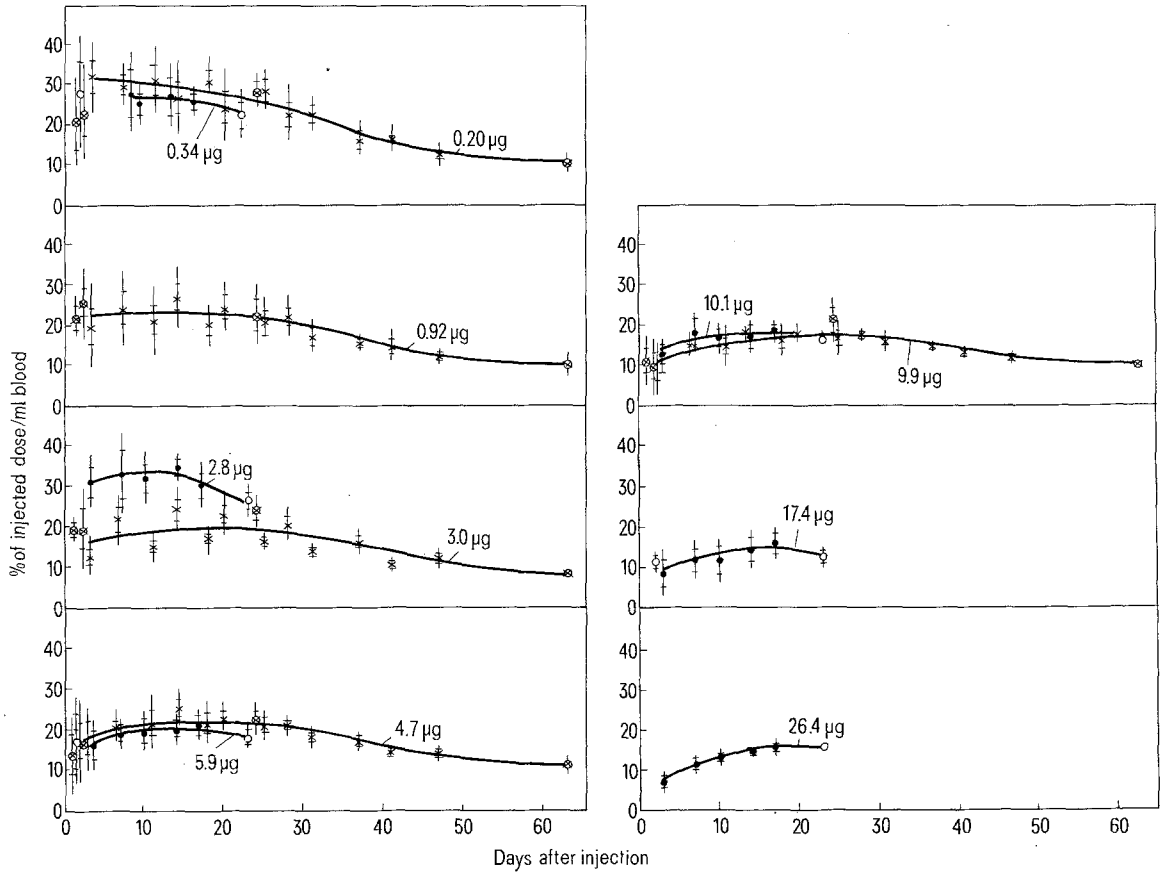
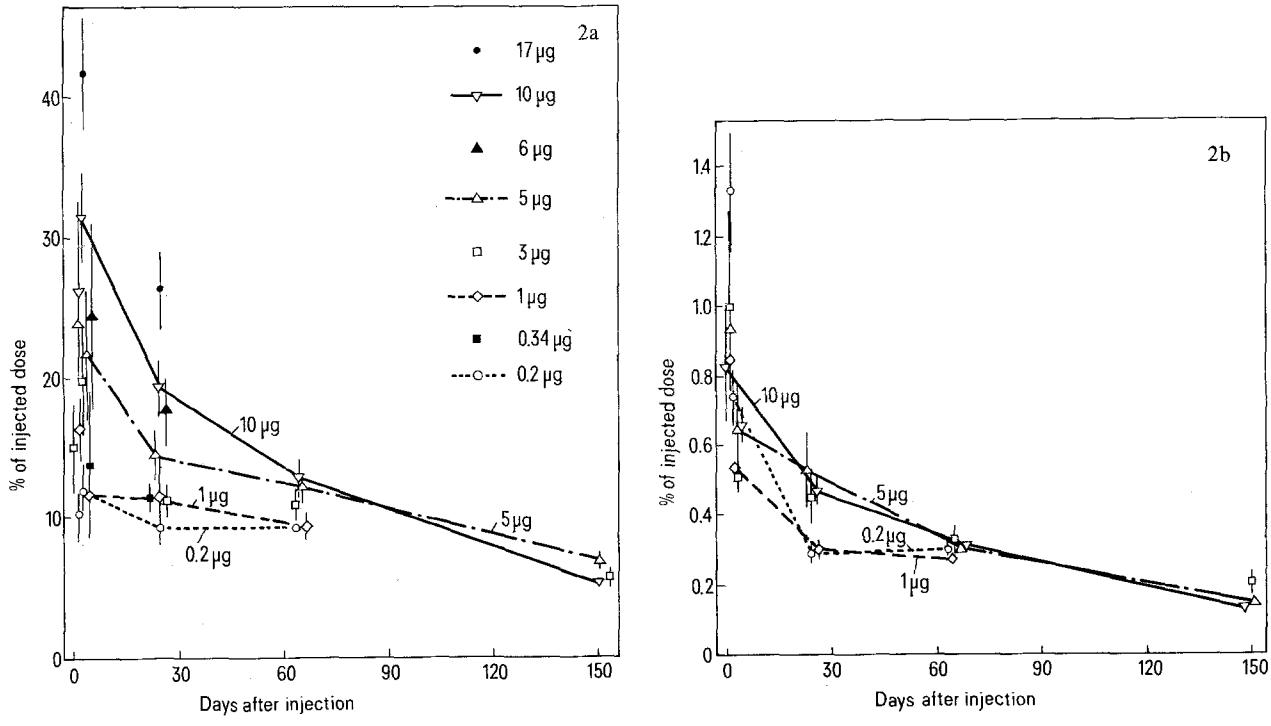


Fig. 1. ^{59}Fe uptake and retention per ml blood. Means (X = experiment 1, 0 = experiment 2), SD (bars), and SE (cross bars) are shown. Weekly retroorbital blood samples of 25 µl were taken from 3–5 mice. Circled symbols denote groups of which 200 µl blood was taken prior to organ sampling. Indicated iron loads are amounts actually measured.



amount of isotope administered but also on the number of stable iron atoms in competition. We used ^{59}Fe instead of ^{55}Fe in a model experiment designed to illustrate and define the degree of competition. Various amounts of stable iron were added to a commercial ^{59}Fe solution. Final solutions contained 25 µCi/ml and from 1 µg to 130 µg Fe/ml as ferric chloride in 0.01 N HCl. Random bred female Swiss albino mice weighing 25–30 g received 0.2 ml isotope i.v. and no other treatment. 2 experiments were performed, one lasted 23 days, the other 150 days. The table shows tissue uptake and retention of the injected ^{59}Fe, measured in whole organs taken from the saline-perfused animals. Each mouse had received 5 µCi in 0.2, 1, 3, 5, or 10 µg of total iron. The influence of concomitant iron load on isotope uptake is plainly visible. This is most explicit in blood, where the greatest iron load is associated with lowest uptake, and in liver where the opposite is true. Dependence of iron uptake on injected total iron also exists in kidneys and hind leg when doses of 0.2 µg or 1 µg are compared with doses higher than 1 µg. With passage of time the remaining isotope in each organ converges towards a common value regardless of initial iron load. This occurs at different speeds in different tissues and requires 7 weeks in blood (figure 1), 9 weeks in hind legs (figure 2, b), and more than 10 weeks in liver and kidneys (figures 2, a and c). The speed of convergence reflects mixing rate of injected iron with body iron. Cellular iron binding and retention as well as vascular iron transport into and out of any given organ govern the rate of mixing. The findings can be explained by reflux of injected iron from initial depositories into other tissues which is known to occur[9–12]. The present experimental approach could be useful in studies of compartmental iron distribution since apparently the time necessary for complete mixing can be well defined for various tissues. Whole body iron retention is not influenced by the injected iron load (figure 3). Thus, the observed organ-specific differences are due to differences in initial iron distribution and not to differences in iron elimination via kidney and intestines.

The data re-emphasize the relevance of specific activity for organ distribution of injected radioiron. Increased liver iron uptake at the cost of bone marrow and blood uptake is also noted when erythroid tissue is diminished by X-irradiation[13], when non-viable red cells serve as iron carrier[9], or when highly saturated transferrin-bound radioiron is injected[14]. Inferences about different affinity of receptor sites on liver cells[14] and reticulocytes[15] for single- and double-iron bound transferrin were not confirmed[16]. Existing iron loads of short[9] or long duration[17] did not impair peripheral blood iron uptake of a tracer dose, even though the plasma iron binding capacity was zero[9]. Most likely, competition between radioactive and stable iron at the transferrin binding site and at the site of the erythron determines the initial isotope uptake in bone marrow and blood. This is suggested by studies on patients[9,10,12] as well as by the present findings. The portion of injected iron not immediately utilized is shunted mainly to the liver.

Most workers inject transferrin-bound or citrate-bound radioiron. We saw radiolysis within 2 h of conversion of high specific activity ^{55}Fe from chloride to citrate and thus preferred to use ionic ^{55}Fe. Immediate shock and death occured after rapid i.v. injection of about 1 µg, but with very slow injection (about 1 µg/min) up to 50 µg ionic iron were tolerated. The carrier HCl solution produced no symptoms. The tracer iron doses of 0.2 µg or 0.34 µg led to blood and tissue uptake comparable to that with citrate iron[13] and transferrin-bound iron[11,14]. Hence, ionic iron can be used conveniently when rapid radiolysis is a risk.

The present results show that ionic iron can be given i.v. in amounts far exceeding the free iron binding capacity of serum. Therefore, relevance of specific activity does not so much concern the feasibility as it does the reproducibility of ^{55}Fe erythrocytocide. Reproducible conditions exist when injected iron does not exceed the free iron binding capacity of 1 µg/ml serum. The highest obtained ^{55}Fe specific activity of 1300 µCi/µg Fe allowed to inject initially up to 700 µCi to a 20 g mouse without exceeding iron binding capacity. One half-life later half the dose would be

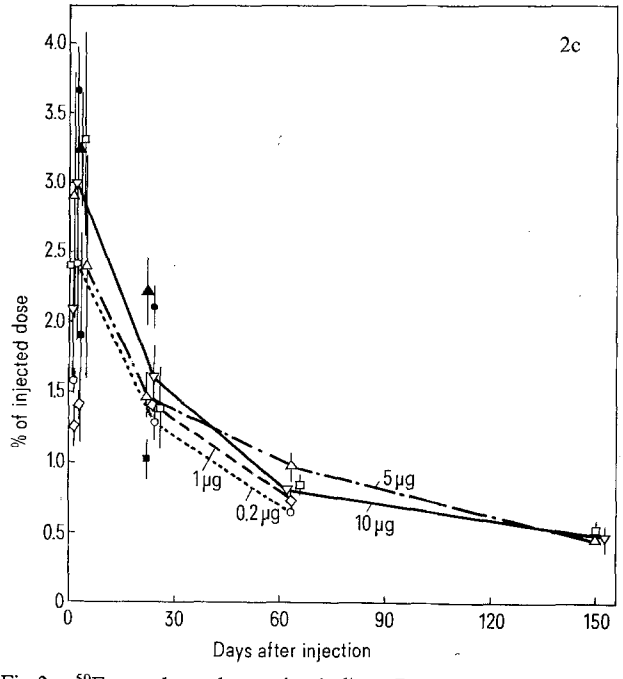


Fig. 2. *a* ^{59}Fe uptake and retention in liver. Data from experiment 2 are line-connected between day 2 and 150. *b* ^{59}Fe uptake and retention in hind leg, i.e. 1 whole, cleaned femur, tibia, and fibula. *c* ^{59}Fe uptake and retention in 2 whole kidneys.

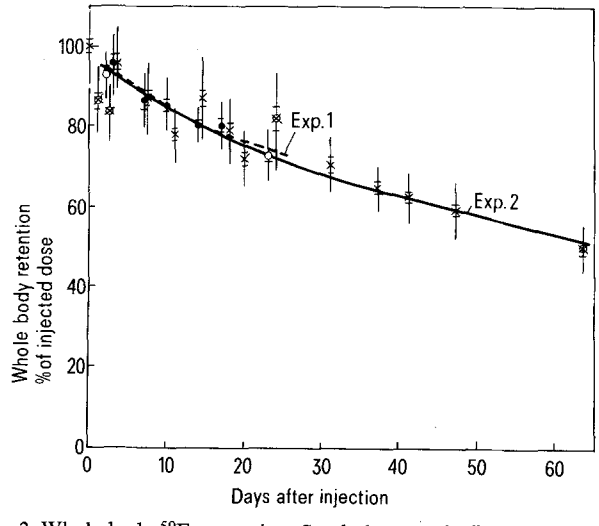


Fig. 3. Whole body ^{59}Fe retention. Symbols are as in figure 1. Each mouse, immobilized inside a small plastic cylinder, was placed on a platform between 2 NaI crystals, one on top and one below in a shielded counter (Tobor, NEN). The data were pooled for each of 2 experiments since there were no significant differences according to iron load. The eye-fitted curve suggests initially increased excretion with about 42 days half-time (1.2%/day) and final excretion with about 90 days half-time (ca. 0.56%/day).

considered reproducible. These calculations illustrate the importance of high specific activity ^{55}Fe for cytocidal studies. They also emphasize the necessity of exact analysis of iron content in samples which conventionally may be designated as 'carrier-free'.

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A new method for measuring radioactivity in select areas of intact tissue sections[1]

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Summary. A new technique is described for measuring radioactivity in select areas of intact tissue sections. The method, which makes use of a liquid scintillation counter, also makes possible identification of the studied zones by prestaining.

Incorporation of labelled compounds into different areas of tissue sections may be assessed by 2 common techniques. 1 of these utilizes a liquid scintillation counter and requires cutting off samples from the sections[3]. The other is the prolonged method of autoradiography[4]. This report describes a new scintillation technique for determining the level of radioactivity in select areas of intact sections.

Materials and methods. Male albino rats were injected into the left brain ventricle with 100 µCi ^{35}S-methionine. Injected brains were removed 30 min later and coronal sections of 8, 16, 32, 64 and 128 µm thickness were sliced in cryostat, mounted on pieces of glass slide and then fixed in Carnoy's fluid (3 parts ethyl alcohol + 1 part acetic acid).

A) For counting the radioactivity in select areas, slides bearing sections 64 µm thick were covered with transparent paper and the select areas were marked off by pencil. This paper was then fastened onto aluminium foil and apertures of 0.5 mm diameter were bored with appropriately sharpened syringe needle at the points indicated on the paper. The perforated foil was now placed on the section-bearing slide with the apertures occurring at the pre-selected areas. The back of the slide was covered by a continuation of the same piece of foil. To expose only 1 select area through 1 aperture in the foil, a solid aluminium plate with a single aperture was used (figure 1). This assembly, comprising the slide, section, foil and plate all held together by an ordinary paper clip, was transferred into a Tri-Carb liquid scintillation spectrometer (Packard), vial filled with scintillator (Insta-Gel, Packard). To measure radioactivity in other regions of the section, the aluminium plate was shifted each time to expose a new area for counting. Identification of histological structures in the specimen was made possible by staining with hematoxylin-eosin, Feulgen, Giemsa or other stains.

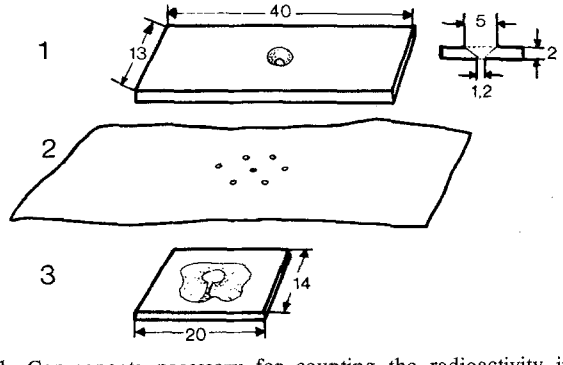

Fig. 1. Components necessary for counting the radioactivity in select areas of the tissue sections by liquid scintillation spectrometer (sizes in mm). *1* Aluminium plate. *2* Aluminium foil. *3* Section on glass slide.

B) Absorption of radioactivity by sections of different thickness was evaluated by comparing the radiation level of the intact section with that following its solubilization. The former was determined by use of a vial containing 4 ml of scintillator and 1 ml of tissue solubilizer (Soluene 350, Packard); the solubilizer, although inactive at such a ratio, was added to yield the same concentration of scintillator molecules as in the section undergoing solubilization. Solubilization of the sections was effected by breaking the slides and immersing the resulting fragments for 3 days at 37 °C in 0.9 ml of tissue solubilizer and 0.1 ml distilled water. The vials were shaken throughout to enable penetration of the solution between the broken pieces and the release of air bubbles. At the end of the incubation, 4 ml of scintillator were added to each vial. For the various thicknesses of sections, the ratio of radioactivity level of the intact section to that of the solubilized one was expressed in percent.

Results. For purposes of illustration of the technique, the values of the radioactivity measured in some areas of the rat brain are presented in the table. As figure 2a indicates, the correlation between the radioactivity of the solubilized