METHODS

A New Method for Evaluation of Radioactive Label Transport Intensity in the Predominant Direction between Blood and Liver

Yu. A. Petrovich, R. P. Podorozhnaya*, and S. M. Kichenko

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The intensity of ^{75}Se transport in the predominant direction after intraperitoneal injection of [^{75}Se]selenate was compared in 1- and 3-month-old rats receiving common vivarium ration or sucrose diet. The incorporation percent, blood/liver relative radioactivity, and relative radioactivity difference coefficient were evaluated in the blood and liver. The dynamics of label incorporation in the blood of rats fed common diets has two peaks (at 1-3 h and 12-24 h) and a drop at 6 h. Coefficient of difference in 1-month-old rats was characterized by a greater amplitude of fluctuations than in 3-month-old animals.

Key Words: *[^{75}Se]selenate transport; blood; liver; diets; rat age*

New methods for evaluation of compounds and atom transport can provide useful data for laboratory experiments and clinical diagnosis.

The study of the transport of Se compounds is important [1,2,4,5,8]. The time of biological half-life of Se compounds in rat liver changes with age [6]; Se modifies the blood glucose level, gluconeogenesis intensity, and glycogenolysis in the liver of intact rats, in humans with diabetes and in rats with streptosotocin diabetes [7,9-12]. However, there are no precise data on the intensity of Se transport between the blood and liver in the predominant direction during certain periods of time, which can be valuable for experimental and clinical medicine.

The aim of this study was to develop a new method for evaluating the dynamics of ^{75}Se transport intensity in the predominant direction from the blood into the liver and vice versa. Age-specific differences in Se metabolism and relationship between carbohydrate and Se metabolism were studied.

Moscow State Medical Stomatological University; *Institute of dentistry, Academy of Medical Sciences of Ukraine, Odessa. ***Address for correspondence:*** kichenko@metronet.ru. S. M. Kichenko

MATERIALS AND METHODS

Total ^{75}Se levels in liver homogenates and blood of rats 1, 3, 6, 12, 24, 48, and 192 h after intraperitoneal injection of [^{75}Se] sodium selenate (20,000 cpm/g) were evaluated by γ-irradiation of the sum of Se-containing compounds using a radiometer with a scintillation pickup with a Tl-activated KI crystal. The percent of ^{75}Se incorporation was estimated by the ratio of cpm/g tissue or 1 ml blood to cpm/g. The relative radioactivity (RRA) was estimated by the formula **(1)**:

$$\text{RRA} = \frac{\%\text{ of incorporation in liver}}{\%\text{ of incorporation in blood}} \qquad \textbf{(1)}$$

After a certain period after injection of the labeled compound, RRA was estimated by formula 1, and after fixed periods RRA_2, RRA_3, and RRA_n were estimated.

Then the predominant direction of ^{75}Se transport was determined and the difference between the trans-

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port in the predominant direction and the transport in the opposite direction was evaluated by deriving the coefficient of difference (CD_{RRA}) by the formula **(2)**:

$$CD_{RRA}=RRA_{n+1}-RRA_n.$$

$CD_{RRA}>0$ indicated the predominance of the radioisotope transport from the blood into the liver, $CD_{RRA}<0$ ("-") indicated predominance of the radioisotope release from the liver into the blood.

The rat pups were breast-fed; starting from week 3 they were transferred to common vivarium fodder. Group 1 rat pups were taken into experiment at the age of 1 month. Group 2 animals received common vivarium rations during subsequent 2 months and were sacrificed at the age of 3 months. Group 3 animals received sucrose diet at the age of 1-3 months. Sucrose diet was as follows: 54% sucrose, 18.5% purified alimentary casein, 18.5% dry wheat bread, 5% sunflower oil, polyvitamin complex, 4% salt mixture (100 g NaCl, 3 g $CaCl_2$; KH_2PO_4, $MgSO_4$, and KCl, 5 g each; $CuSO_4$, $ZnSO_4$, $Co(NO_3)_2$, and $FeCl_3$, 0.01 g each).

The results were processed using Student's *t* test.

RESULTS

In group 1 the percent of label incorporation in the liver and blood 1, 3, 24, 48, and 192 h after injection was significantly lower than in adult rats (groups 2 and 3, Fig. 1, *b*, *c*). Six hours after injection the incorporation percent was higher in rat pups ($p<0.05$).

The maximum RRA levels were recorded 6 h after injection in rats and rat pups fed sucrose diet. In 3-month-old rats receiving common ration the RRA peaked as early as 3 h postinjection.

The curve presenting changes in the percent of label incorporation in the blood (Fig. 1, *c*) has 2 peaks in all animals: during the first 3 h after injection (followed by an appreciable drop by 6th hour) and second peak by hours 12-24, sharply pronounced only in rats

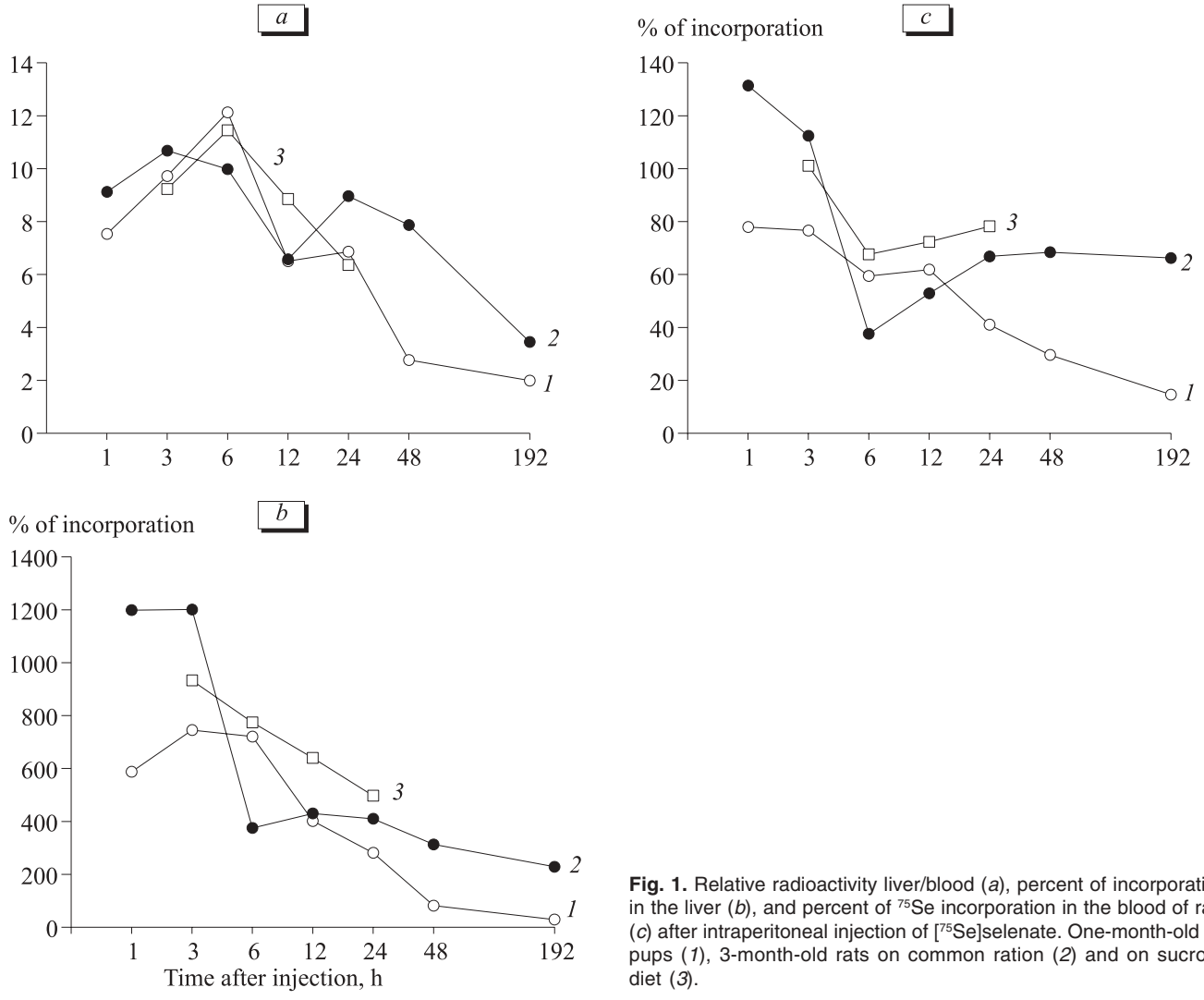

Fig. 1. Relative radioactivity liver/blood (*a*), percent of incorporation in the liver (*b*), and percent of ^{75}Se incorporation in the blood of rats (*c*) after intraperitoneal injection of [^{75}Se]selenate. One-month-old rat pups (*1*), 3-month-old rats on common ration (*2*) and on sucrose diet (*3*).

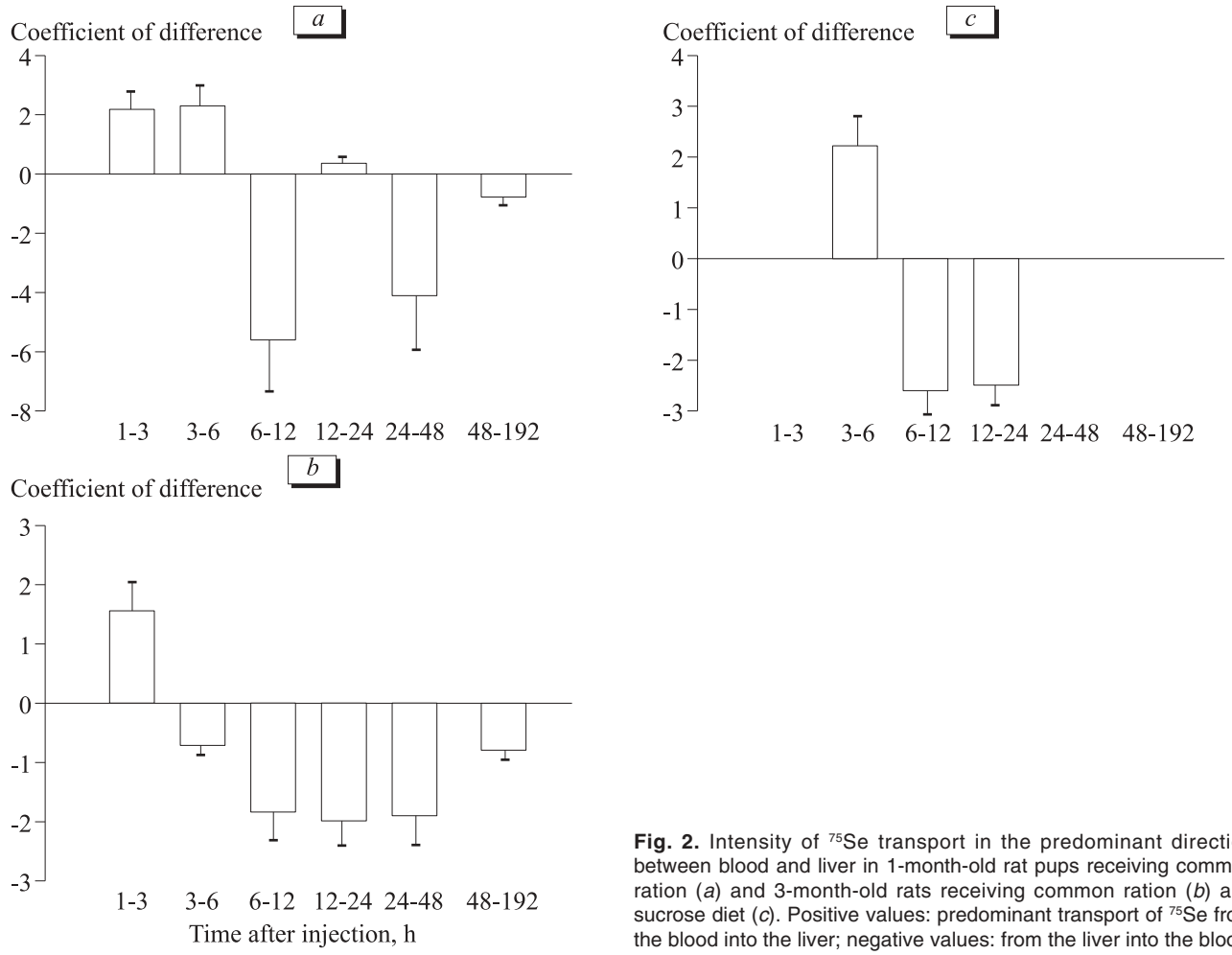

Fig. 2. Intensity of ^{75}Se transport in the predominant direction between blood and liver in 1-month-old rat pups receiving common ration (*a*) and 3-month-old rats receiving common ration (*b*) and sucrose diet (*c*). Positive values: predominant transport of ^{75}Se from the blood into the liver; negative values: from the liver into the blood.

fed common ration. In the liver a trend to an increase in the percent of ^{75}Se incorporation was observed only in animals receiving common ration. Rats receiving common ration also exhibited RRA with the peak at 24 h also acquired a clear-cut "two-peak" pattern. A trend to a slight increase by this term was observed in rat pups.

The two-peak curve can be due to primary labile absorption of the label on proteins and subsequent rapid desorption. Presumably, the appearance of the second peak is due to stronger chemosorption of [^{75}Se]selenate and its metabolites; their formation after 24 h and more after injection of the labeled compound cannot be completely ruled out. A similar two-hun-

TABLE 1. Intensity of ^{75}Se Transport in Predominant Direction from Blood into Liver or from Liver into Blood (Negative CD_{RRA} Value) after Intraperitoneal Injection of [^{75}Se]Selenate

Parameter		Hour postinjection						
		1	3	6	12	24	48	192
1-month-old rat pups	RRA	7.54	9.72	12.13	6.50	6.86	2.77	1.99
	CD_{RRA}		2.18	2.39	-5.60	0.36	-4.11	-0.78
3-month-old rats on sucrose diet	RRA	9.12	10.68	9.97	8.13	6.14	4.24	3.45
	CD_{RRA}		1.56	-0.71	-1.84	-1.99	-1.90	-0.79
3-month-old rats on common ration	RRA		9.23	11.45	8.85	6.36		
	CD_{RRA}			2.22	-2.60	2.49		

ched curve of labeled carbonate metabolism in bones was observed after bone fracture [3].

Using formula 2, CD_{RRA} of [^{75}Se]selenate transport intensity in the predominant direction between the blood and liver during certain periods was calculated. CD_{RRA} was fixed for 6 terms: 1-3, 3-6, 6-12, 12-24, 24-48, 48-192 h after injection of the radioisotope.

The range of CD_{RRA} fluctuations in 1-month-old rats surpasses the shifts in this parameter in 3-month-old rats, which can be due to more intense metabolism in the rat pups (Fig. 2). CD_{RRA} at first increased and then decreased in all groups. In 1-month-old rat pups the maximum CD_{RRA} was observed during the period of 3-6 h, while in 3-month-old rats receiving common ration the peak was observed during 1-3 h.

CD_{RRA} was calculated by two methods, depending on the trend of RRA changes: if the direction of RRA changes was the same, lesser RRA value was subtracted from the greater one, while if RRA shifts were opposite, the coefficients were summed.

For example, RRA increased in 3-month-old rats receiving common ration (Table 1; CD_{RRA} 1.56) and in 1-month-old rat pups (CD_{RRA} 2.18). Subtraction of 1.56 from 2.18 shows that the radioisotope transport from the blood into the liver in the rat pups is 40% higher than its transition from the liver into the blood in 3-month-old rats receiving common vivarium ration.

Hence, the intensity of the label transport in the predominant direction during a certain period can be evaluated by changes in CD_{RRA}.

Using this method, it is possible to determine the intensity of predominant direction of transport of not only labeled radioactive isotopes, but of labeled heavy stable D, ^{15}N, ^{18}O, and other isotopes from the blood into the liver or vice versa and quantitatively characterize the intensity of transport of an unlabeled substance, if it was not present in the body before injection.

The preposed method for rapid demonstrative determination of the predominant transport of the label from the blood into the liver or vice versa can be used for other organs.

Quantitative evaluation of the time course of RRA and CD_{RRA} can be used in oncology when selecting a radioisotope for the diagnosis (if the malignant tumors accumulate more radioisotope in the tumor zone and metastases than benign tumors). Radioisotopes with higher CD_{RRA} with predominant transport from the blood into the involved organ are preferable for the diagnosis and treatment.

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