Liver homing in the rat of ^{111}Indium labelled beef and neuraminidase-treated rat erythrocytes (Studies with a Gamma Camera)

E. Friedrich, R. Süss, D. Hock, G. Uhlenbruck[1], R. Kubesch and H. Sinn[2]

Deutsches Krebsforschungszentrum Heidelberg, Im Neuenheimer Feld 280, D-6900 Heidelberg 1 (Federal Republic of Germany), 7 February 1977

Summary. The rate of liver homing of ^{111}In-labelled erythrocytes has been measured under a Gamma Camera. Homing of neuraminidase-treated or xenogeneic erythrocytes is delayed by preinjection of glycolipids or glycopeptides.

Neuraminidase-treated erythrocytes are cleared by the liver when reinjected into rats[3]. Sheep red blood cells[4] and other xenogeneic erythrocytes (Friedrich, unpublished) 'home' to the rat liver without pre-treatment. These sequestration processes may be triggered by surface components, 'garbage signals' exposed by the enzyme treatment or permanently available on xenogeneic cells. Ashwell's experiments with desialiated glycoproteins suggest that terminal sugars may mediate liver trapping[5]. Thus liver homing of erythrocytes should be delayed by preinjecting appropriate sugar-containing molecules. In this paper we report on some experiments which show that glycolipids delay sequestration of neuraminidase-treated rat red blood cells and that polypeptides isolated from bovine erythrocytes delay homing of bovine erythrocytes to rat liver.

Bovine erythrocytes were washed in saline, rat erythrocytes were incubated with neuraminidase from Vibrio cholerae (Serva) for 1 h (100 µl packed erythrocytes with 5×10^{-3} units). Erythrocytes were labelled with ^{111}Indium as described[6] and injected through the tail vein (0.25 ml packed erythrocytes in a total volume of 0.5 ml saline). Glycopeptides isolated from bovine erythrocytes[7] were dissolved in 1 ml saline (100 mg/ml) and injected 3 min before the injection of labelled erythrocytes. Gangliosides (total fraction of 'acid glycolipids') were isolated from beef brain[8], suspended in saline by ultra sonication (5 or 10 mg/ml) and 1 ml injected again 3 min before the labelled erythrocytes. For the purpose of comparison 1 ml of the following solutions were preinjected: galactose (0.4 M), methylglucose (0.4 M) and bovine albumine (10 mg/ml).

The anaesthetized rats were scintigraphed with a Gamma Camera (Nuclear Chicago) and the data stored by a computer. Figure 1 shows an animal scintigram: The counts accumulated up to 2 min clearly show circulating erythrocytes, after 30 min most of the radioactivity is trapped in the liver. For the quantitative studies 2 'regions of interest' were selected. A 'heart region' as

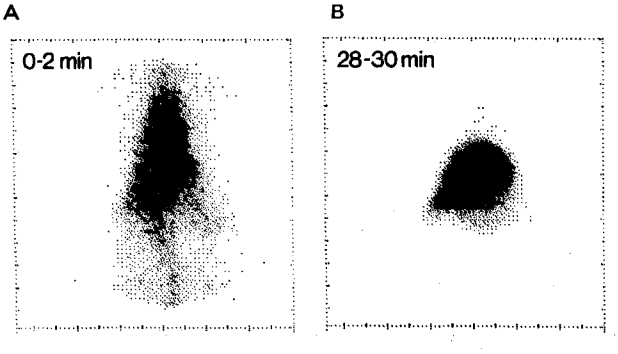

Fig. 1. Whole animal ^{111}In scintigram. 0.5 ml Indium labelled erythrocytes (neuraminidase-treated rat erythrocytes) injected intravenously. A. Scintigram between 0 and 2 min; B. Scintigram between 28 and 30 min.

1 Medizinische Klinik, Universität Köln (Federal Republic of Germany).
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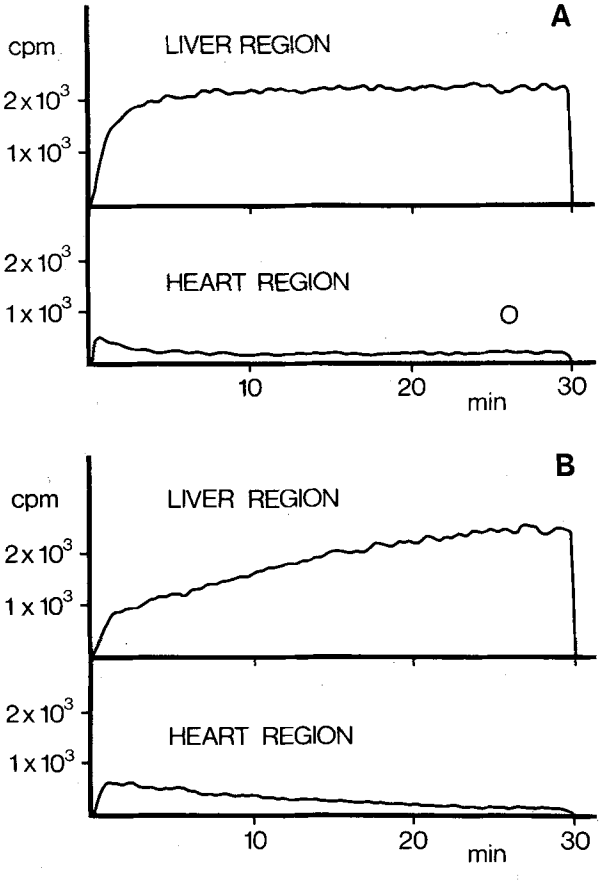

Fig. 2. Time-dependent radioactivity detected in specified liver and heart regions (cpm/region). Heart region as a measure of circulating radioactivity. Liver region representing the storing organ. Neuraminidase-treated rat erythrocytes without (A) and with (B) preinjected glycolipid fraction.

representative for circulating radioactivity and a 'liver region' representative for the storing organ. Figure 2 shows the time course of radioactivity detected in these 2 defined regions: A. Sequestration of neuraminidase-treated erythrocytes is completed after 6 min. Increase in the liver region and decrease in the heart region are closely correlated. B. After preinjection of glycolipids sequestration of neuraminidase-treated erythrocytes is delayed by a factor of about 3. Here again, the decrease in the heart region corresponds with the increase in the liver region. The results of experiments with different erythrocytes and different competing agents are listed in the table. The observed homing of erythrocytes, either neuraminidase-treated or xenogeneic is irreversible: after 24 and 48 h the radioactivity is still localized in the liver region (the term 'last homing' would therefore be more appropriate). It is probable that the erythrocytes reach the liver as intact cells: a) in vitro incubation of the used erythrocytes with rat serum did not lead to fragmentation. b) Another argument comes from homing experiments with lysed ^{111}Indium labelled erythrocytes. When this lysate was injected into rats, the radioactivity stayed first in circulation, slowly accumulating in the kidneys rather than in the liver. (In this lysate Indium radioactivity is associated to more than 95% with hemoglobin)[9]. This may indicate that surface components are recognized by liver cells. Preinjected glycolipids and glycoproteins may block presumptive recognition sites. This is in line with Ashwell's experiments; however, there seems to be a major difference: Desialiated glycoproteins are obviously trapped and incorporated by liver parenchymal cells[5] whereas erythrocytes homing to liver appear to be taken up by sinusoidal cells[10]. Thus it may be concluded that RES cells of the liver recognize altered or xenogeneic cells by surface components. This could be relevant not only for the elimination of erythrocytes. Tumor cells could show similar signs of 'foreigness' and indeed, tumor cells have been found to home to liver irreversibly[4,11]. The functional integrity of liver sinusoidal cells may thus play an important part in the control of tumor cell populations.

Liver sequestration kinetics

Erythrocytes and competing agents	Time for maximal sequestration	
Rat erythrocytes neuraminidase-treated	5 ± 1.5	(7)
Gangliosides (5 mg/animal)	14 ± 4	(4)
(10 mg/animal)	25 ± 5	(2)
Methylglucose	> 15	(2)
Galactose	> 15	(2)
Bovine erythrocytes	18 ± 4	(6)
Glycopeptides (10 mg/animal	> 30	(4)
Bovine albumine (10 mg/animal)	15 ± 5	(3)

The agents mentioned were preinjected 3 min before injection of the labelled erythrocytes. Details see text. Number of animals given in parentheses.

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Changes in the T_4/T_3 molar ratio following thyrotrophin releasing hormone injection in cattle

A. B. Ślebodziński and A. L. C. Wallace

Polish Academy of Sciences, Department of Experimental Pathology of Animals, ul Grunwaldyka 250, 60–166 Poznan (Poland), and CSIRO, Division of Animal Production, Prospect, N. S. W. 2148 (Australia), 10 January 1977

Summary. The injection of thyrotropin releasing hormone into cattle resulted in a rapid decrease in the T_4/T_3 molar ratio. 2 breeds of cattle, Shorthorn and Africander Cross were studied. The decrease in the T_4/T_3 molar ratio was significantly greater in the Shorthorn breed. It is concluded that acute stimulation of the thyroid gland with TRH results in enhanced release of both T_3 and T_4 and that T_3 is discharged more rapidly than T_4.

There are marked differences among species in the ratio of thyroxine (T_4) to triiodothyronine (T_3) in the thyroid gland. Thus the T_4/T_3 ratio in man has been reported to be 20:1[1] and in rat 6:1[2]. Since there appears to be only very limited conversion of T_4 to T_3 in the thyroid[3,4] it is reasonable to expect that, in general, T_3 and T_4 will be secreted in the same ratio as they occur in the gland. However, little is known about the variation of the thyroid T_4/T_3 ratio within a particular species or of conditions which may alter the secretion rate of T_4 to T_3. In this study we report acute changes in the serum T_4/T_3 molar ratio in 2 breeds of cattle following the injection of TRH.

Methods. 2 groups of 6 cattle, one comprised of Shorthorns (SH), a temperate climate adapted breed and the other of Africander Crosses (AX), a tropically adapted breed, were injected i.v. with TRH (Calbiochem, Lot 30075). 3 injection schedules were used: a) a single injection of either 0.4, 1, 2.5 or 5 μg/kg live weight (l.wt), b) 2 injections of 3 μg/kg l.wt 24 h apart or c) repeated injections of increasing amounts of TRH (1, 2, 3, 4 and 5 μg/kg l.wt) hourly over 4 h. This latter injection schedule gave a total dose of 15 μg/kg l.wt per animal. Blood samples were taken at intervals from a jugular vein and the resulting serum samples analyzed using radioimmunoassays for thyroid stimulating hormone (TSH), T_3 and T_4. Experiments were carried out at least 1 week apart to allow a recovery period before the next injection of TRH.

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