Studies on the Turn-Over and Distribution Between Plasma and Blood Cells of Radioactive Palmitate in Pregnant Rats

Using the tracer experiment in a compartmental system for the estimation of rate constants, precise data of the specific activity changes of the initial compartment are required. If radioactive palmitate is used as tracer for free fatty acids (FFA) and the initial compartment is the plasma, at zero time the determination of the specific activity is not possible, because in physiological research the condition of uniform mixing of label does not exist at zero time. It has been commonly held[1] that the value at zero time obtained by extrapolation is taken for 100% of injected radioactivity; but the extrapolated value is not related to measurments of the actual injected radioactivity.

We could not account for a certain fraction of i.v. injected labeled FFA when extrapolating the data to zero time and found that this missing fraction of radioactivity was present in the blood cells. The present study was conducted to determine the proportion of i.v. injected labelled FFA entering the blood cells.

Methods. The plasma volume of pregnant Wistar rats (21 days) was determined by Evans blue[2]. Pregnant Wistar rats (21 days) were anesthetized and albumin-bound radioactive palmitate injected into the tail vein. At intervals from 0.2 min to 0.7 min after the injection, venous capillary blood was collected from the ophthalmic sinus. The serum was extracted for lipids according to the method of FOLCH et al. and assayed for radioactivity. The disappearance rate of injected palmitate was estimated by linear regression analysis. Details of the methods used have already been described[3]. At 0.4 min to 0.6 min after the injection of label, blood was also collected from the vena cava inferior using a syringe containing sodium citrate. The blood was centrifuged, and the serum saved. The serum trapped in the blood cells was recovered by repeated washing with physiological saline. The blood cells were hemolyzed in water. Samples of blood, serum, salines and blood cells were dissolved in Hyamine, toluene-counting solution was added, counted in a Packard scintillation counter, and corrected for quenching.

Another aliquot of the washed blood cells was extracted for lipids with chloroform-methanol (2:1, v/v) for 48 h, and washed with water. The lipid extract was separated by thin layer chromatography, and the radioactivity of the individual fractions was determined. Another aliquot of the washed blood cells was suspended in serum (1:1, v/v) of pregnant rats, incubated at 37 °C for 1 min, centrifuged, and the serum was saved. The residual serum was recovered by repeated washing with physiological saline. The radioactivity in the serum and salines were determined (see above).

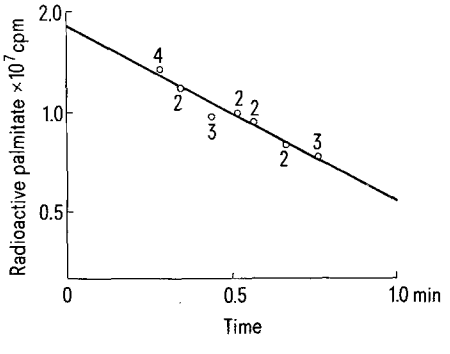
Results. The plasma volume of pregnant Wistar rats (21 days) is 4.3 ± 0.3 ml per 100 g body weight. The Figure shows the disappearance curve of radioactive palmitate from plasma. After the injection of (20 ± 0.4) $\times 10^6$ cpm ($=C_o$) the disappearance curve starts at zero time. The extrapolated value for C_o is (17 ± 2) $\times 10^6$ cpm. The distribution of label in the blood at 0.4 min to 0.6 min after injection of label is represented in the Table. Of the total radioactivity in blood cells 80 ± 15% are recovered in the FFA fraction, and 20 ± 4% ($n = 4$) in the phospholipid fraction. After incubating blood cells and serum, 70 ± 6% ($n = 3$) of the total radioactivity present in blood cells are recovered in serum.

Discussion. Intravenously injected radioactive palmitate is very rapidly eliminated from the circulation of pregnant rats. The initial slope of the disappearance curve follows a simple exponential function during the first minute (Figure). This finding was also reported for non-pregnant rats[4,5]. When extrapolating the curve to zero time, we found only 85% of the injected label in the serum. Two points could qualify for the missing radioactivity. First, during injection a fraction of injected label disappears from plasma at the turn-over rate, and second, some exchange of label is considered possible between blood cells FFA and plasma FFA[6]. The duration of injection (Δt) ranged from 4 to 5 sec. The loss of radioactivity (ΔC) occurring during injection can be approximated as $\Delta C = \frac{\lambda \Delta t}{2} \times 100\%$ of the injected label. The loss amounts to 5%. 9% of the injected label could be shown to be present in blood cells, which could not

Distribution of radioactivity in blood after the injection of radioactive palmitate into the tail vein of pregnant rats

Sample	Recovered radioactivity
Serum	82 ± 4
1. 0.9% saline wash	9 ± 1.6
2. 0.9% saline wash	0.8 ± 0.2
Blood cells	9 ± 0.9
Total recovery	100.8

The radioactivity recovered in each sample is expressed as percentage of radioactivity found in whole blood (= 100%). Values are shown as means ± S.D. of 7 experiments.



Disappearance curve of tracer from maternal plasma after injection of palmitate-1-^{14}C. The fractional turnover rate λ was estimated by linear regression to be 1.2 ± 0.12 min^{-1} (mean ± standard error). Each value represents the mean of 2 to 4 estimations. The number of estimations is indicated in the figure. Data, which are plotted on a semilogarithmic scale, are obtained from 5 rats.

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be removed by washing with 0.9% saline. $^1/_5$ of the label present in blood cells was incorporated in the phospholipid fraction. The bulk of label was recovered in the FFA fraction of blood cells, which was shown to be exchangeable with the plasma FFA.

The results are interpreted to indicate that 1 FFA compartment[6] of blood cells[8] is thought to have so rapid a turnover that the FFA compartment of the plasma and blood cells may be considered as one compartment, which is greater than the FFA compartment of the plasma FFA. Thus, quantitative considerations of the fate of injected label must be emphasized in order to avoid spurious low values for the turn-over rate of plasma FFA.

The FFA concentration of 21 days pregnant rats maintained under light ether anesthesia for approximately 7 min was found to be 0.64 μM/ml plasma[7].

Zusammenfassung. 20 sec nach i.v. Injektion radioaktiven Palmitates findet man 9% der Radioaktivität in den Blutzellen, wo sie zu 80% in der FFS-Fraktion nachgewiesen wurde. Das radioaktive Palmitat kann durch Serum, nicht aber durch physiologische Kochsalzlösung aus den Blutzellen entfernt werden. Bei Nichtbeachten dieser Befunde wird die Umsatzrate der FFS des Serums zu niedrig bestimmt.

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DNA-Feulgen Value in Brain Cells of the Adult Worker Honeybee Dependent on Age[1]

The question of whether a correlation exists between DNA content per nucleus and age of bees was investigated. The investigations covered the lifespan from the emergence to 30 days of adult life.

One brood comb was placed in a thermostaticly controlled incubator at 35 °C. The young bees that hatched in the incubator were marked with paint. Most of the bees were restored to the beehive while approximately 70 were maintained in a wire mesh cage (25 × 7 × 3 cm) within the hive, thereby prohibiting normal age-dependent behavior[2].

The heads of the bees, 0, 2, 4, 6, 8, 10, 12, 15, 18, 22, 26 and 30 days of age, were dissected, stored in 10% glycerol which was immediately frozen in fluid nitrogen (−196 °C). Some days later, the heads were thawed and the brains removed from which the corpora pedunculata (CP) were dissected out. The tissues were fixed for 10 sec in 50% HAc, thereafter for 15 min in 70% alcohol, and hydrolysis for 30 min at room temperature in 5 *n* HCl subsequent to staining for 2 h in the Feulgen solution. In order to obtain a measurable layer of intact round nuclei, the freezing process was essential.

Preparations from 3 bees from each age group were slightly squashed under a siliconized cover slip. The absorption of the DNA-Feulgen complex was measured at 567 nm on 12 smeared nuclei for each CP with a Zeiss microphotometer 01 by the plug method. Diploid nuclei with only approximately equal radii were measured[3,4] which made it necessary to correct the DNA value for each nucleus with the real radius. The mean radius of the nuclei measured was 1.127 ± 0.006 µm. The DNA content of the nuclei must satisfy the criterion that DNA had not been synthesized in preparation for a mitotic division. Earlier investigations have shown no mitotic activity occurs later than 3 days prior to emergence[4,5].

From the results in the Figure, it was observed that in the young bees the DNA values varied widely. The DNA value of bees at 2 days of age showed a 30% lower value compared to newly emerged bees. From the 2nd to the 4th day, the DNA value increased 47%. At the 10th day, a third peak was observed. It is possible that the real minima and maxima of the DNA values lay somewhere between the age groups investigated. Forager bees, 22 to 30 days old, showed a constant DNA value with a deviation less than 3%.

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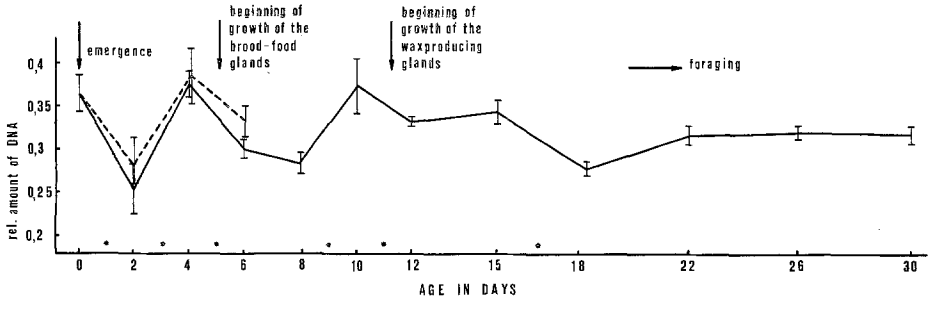

Age dependent DNA-Feulgen values measured in brain cells (CP) of adult worker honeybee. Relative amount of DNA in bees kept under natural conditions (—). Relative amount of DNA in bees maintained in a wire mesh cage (---). Significant differences ($p = < 0.05$) between consecutive age groups indicated by * sign (analysis of variance and Duncan-test). The day of emergence was taken as day 0.