

THE ABSORPTION AND DISTRIBUTION OF 1-¹⁴C-GLYCEROL AND D-³H-GLUCOSE FROM THE REPRODUCTIVE TRACTS OF FEMALE MICE

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OPSOMMING: DIE ABSORPSIE EN VERSPREIDING VAN 1-¹⁴C-GLISEROL EN D-³H-GLUKOSE UIT DIE VROULIKE GESLAGSKANAAL VAN MUISE

'n Draer-oplossing met 2 μ Ci 1-¹⁴C-gliserol en 10 μ Ci D-³H-glukose is in die baarmoeders van muise ingespuut. Die muise is van nul tot vyf uur na die isotoopinspuiting geslag en die geslagskanale en lewers verwyder en geoksideer tot CO₂. Beide ¹⁴C-gliserol en ³H-glukose is vinnig uit die geslagskanaal verplaas (P < 0,01); 97% van die ¹⁴C-gliserol en 96% van ³H-glukose het binne 30 minute na inspuiting uit die geslagskanaal verdwyn. Van die ¹⁴C en ³H kon 58 en 20% respektiewelik op hierdie stadium in die lewer gevind word. Gedurende die oorblywende 4 1/2 uur van monsterneming het die aktiwiteit van ¹⁴C en ³H in die geslagskanaal en lewer skerp afgeneem. Hierdie afname was egter gering in vergelyking met die oorspronklike afname in aktiwiteit. Minder ¹⁴C-gliserol en ³H-glukose is uit die geslagskanaal verplaas tydens estrus as in ander stadia van die geslagsiklus (P < 0,05).

SUMMARY:

Mice were given an intrauterine injection of a carrier solution containing 2 μ Ci of 1-¹⁴C-glycerol and 10 μ Ci of D-³H glucose. The mice were killed from zero to five hours after isotope injection and the reproductive tracts and livers were removed and subsequently oxidized to CO₂ and H₂O. Both ¹⁴C-glycerol and ³H-glucose were rapidly depleted (P < 0,01) in mice reproductive tracts; 97% of the ¹⁴C-glycerol and 96% of the ³H-glucose disappeared from the reproductive tracts 30 minutes after injection. Fifty-eight per cent of the ¹⁴C and 20% of the ³H could be accounted for in the liver in this time. There was a considerable reduction in the activities of ¹⁴C and ³H in the reproductive tracts and livers over the remaining 4 1/2 hour sampling period. However, this reduction was negligible in comparison with the initial decrease in activity. Less ¹⁴C-glycerol and ³H-glucose disappeared from the reproductive tract at oestrus than at other phases of the oestrous cycle (P < 0,05).

There are few reports in the literature concerning the absorption of inseminated compounds from the reproductive tract and their ultimate fate in the body. Mann (1964) reported that certain seminal plasma constituents were barely detectable in the uterine horns of gilts within six hours of insemination. The stage of the oestrous cycle has been shown to influence the metabolic pattern of the rat uterus (Kerly, 1937, 1940; Saldarini, 1967, 1968; Yochim and Saldarini, 1969).

The object of this experiment was to investigate the rate of disappearance of injected ¹⁴C-glycerol and ³H-glucose from the mouse reproductive tract as affected by the stage of the oestrous cycle.

Procedure

Eighty mature female mice were each given an intrauterine injection of 2 μ Ci of 1-¹⁴C-glycerol, Amer-sham and Searle Corp., Des Plaines, Illinois, (specific activity: 15,4 mCi/mM) and 10 μ Ci of D-³H-glucose (specific activity: 792 mCi/mM) in a 0,1 ml "carrier" solution consisting of seven per cent glycerol by volume, 1,0 g of glucose and made up to 100 ml with 0,9% saline. The concentrations of glycerol and glucose used as a "carrier" are similar to those employed in a number of media, which have proved successful in the long-term preservation of bovine semen.

The mice were restrained for isotope injection by holding them at the back of the neck with index finger and thumb and by claspings the tail with the little finger.

A speculum was then placed in the vagina to facilitate the location of the cervical opening. With the aid of the speculum, a blunt 21-gauge needle was passed through the cervix and into the uterus. The isotopes and carrier were then gently expelled into the uterine lumen.

The mice were divided into groups of eight and killed at intervals of 30 minutes over a five-hour period from the time of isotope injection. Vaginal smears were taken from each mouse to establish the stage of the oestrus cycle. The reproductive tracts and livers were removed as rapidly as possible and immediately frozen in separate receptacles.

Upon thawing, the liver of each mouse was weighed and a 0,25 g aliquot (wet weight) was prepared for oxidation as described by Kaartinen (1969). For the purpose of increasing the efficiency of combustion of the tissues, they were wrapped in filter paper prior to burning. The samples were burned in a sample oxidizer*, the ¹⁴CO₂ being collected into one vial and ³H-water into another vial. This sample oxidizer was designed for collecting ³H-water and consequently had to be modified for ¹⁴CO₂ recovery. The modification entailed venting the CO₂ through polyethylene tubing and bubbling it through hyaminéhydroxide (2 ml). The hyamine hydroxide-¹⁴CO₂ complex was then recovered for scintillation counting. The water produced upon combustion was brought into solution with the scintillation solution by the addition of 2-methoxyethanol (4 ml) to each sample. The scintillation solution consisted of 4 g of 2,5-diphenyloxazole (PPO), 50 mg of 1,4-bis

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2-(4-methyl-5-phenyloxazoly) benzene (POPOP) and toluene (1 000 ml). The samples were counted in a liquid scintillation spectrometer* (preset for 20 minutes or 20,000 counts).

The activities of ^{14}C -glycerol and ^3H -glucose for time zero were determined as follows: reproductive tracts were removed from three untreated mice and injected with $2\mu\text{Ci}$ of $1\text{-}^{14}\text{C}$ -glycerol and $10\mu\text{Ci}$ of $\text{D-5-}^3\text{H}$ -glucose. The reproductive tracts were then oxidized and the ^{14}C and ^3H collected as previously described.

The recovery and counting efficiencies were maintained constant for all samples tested. In view of this constancy, the results of this experiment are presented in counts per minute (cpm) less background and were not corrected for tissue weight.

Results and Discussion

The rate of disappearance of ^{14}C -Glycerol and ^3H -Glucose from the mouse reproductive tract

The results of this phase of the experiment are presented in Fig. 1. Figure 1 indicates a very rapid decline in the activities of ^{14}C and ^3H within the reproductive tract within 30 minutes of isotope injection ($P < 0.01$). Apart from the initial decrease in the activities of ^{14}C -glycerol and ^3H -glucose, it was observed that the activities of these compounds in the reproductive tract fluctuated in a proportionate manner throughout the remainder of the sampling period. The reduction

in the activities over the remaining $4\frac{1}{2}$ hour period from the time of injection was considerable, but negligible in comparison with the initial decrease in activity. The decrease in the activities of ^{14}C -glycerol and ^3H -glucose in the reproductive tract within 30 minutes after injection was 97% and 96%, respectively. This suggests that glycerol and glucose disappear or are absorbed at approximately the same rate from the reproductive tract and do not appear to be differentially absorbed.

The activities of ^{14}C and ^3H in the liver in the first 30 minutes accounted for 58 per cent of the injected ^{14}C and 20 per cent of the injected ^3H . The ^{14}C and ^3H did not decline to any appreciable extent over the remaining $4\frac{1}{2}$ sampling period. The activities of ^{14}C -glycerol and ^3H -glucose in the reproductive tract decreased significantly between 30 and 90 minutes after injection ($P < 0.01$). However, there was a sharp increase in ^{14}C and ^3H over the next 90 minutes followed by a decrease in activity over the remainder of the sampling period.

The very rapid disappearance of ^{14}C -glycerol and ^3H -glucose from the reproductive tract and the accumulation of 20% of the ^3H and 58% of the ^{14}C in the liver poses the question as to where 80% of the ^3H -glucose and 42% of the ^{14}C -glycerol which were unaccounted for, has disappeared to. It could be speculated that these compounds were rapidly absorbed and carried to other body tissues by the general circulation and were then evenly distributed through-

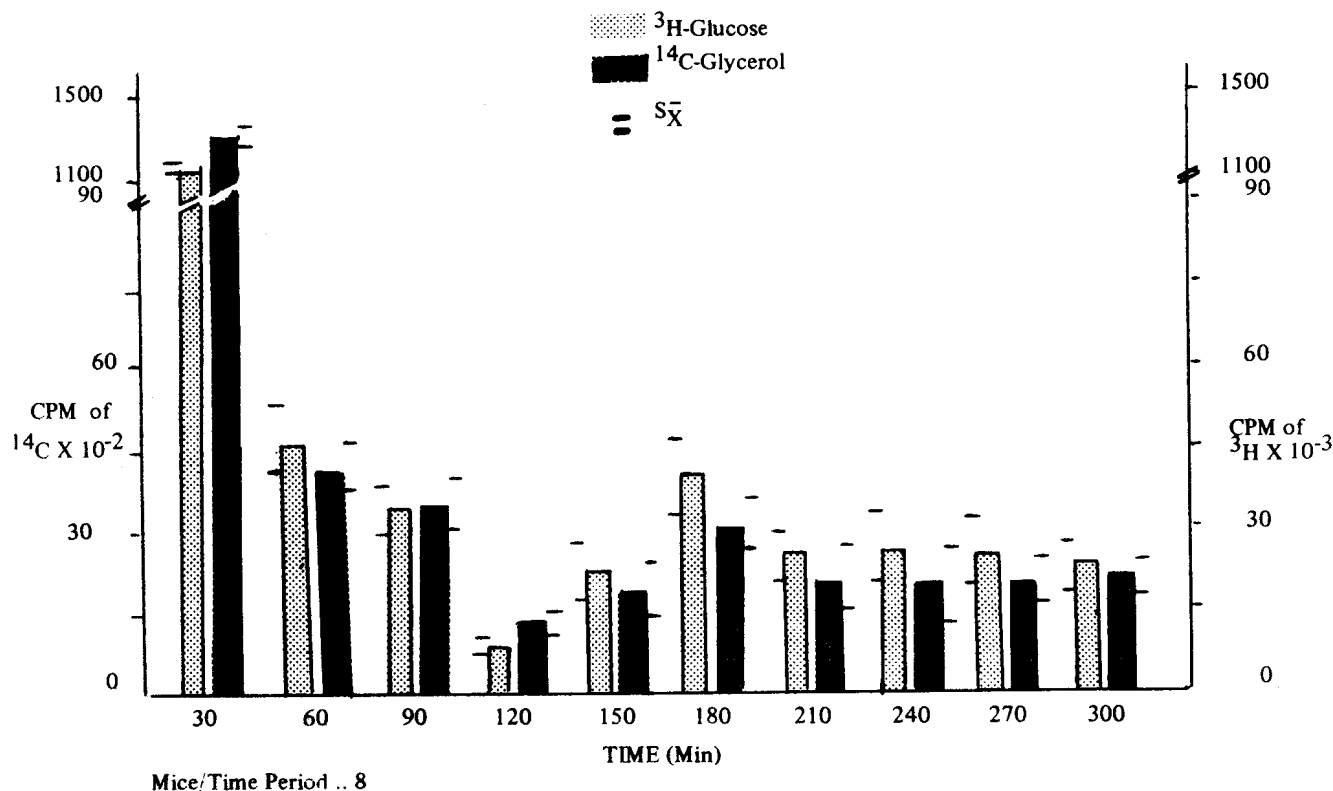


Fig. 1 The level of $1\text{-}^{14}\text{C}$ -Glycerol and $\text{D-5-}^3\text{H}$ -Glucose in mice tracts as affected by time.

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out the body. These results are in agreement with the findings of Mann (1964) who reported that seminal plasma constituents were almost entirely removed from the uterus within six hours of insemination.

The physiological significance of the finding that ^{14}C -glycerol and ^3H -glucose rapidly disappear from the reproductive tract after injection is not entirely clear from these data. However, it does suggest that glycerol and glucose are expelled or absorbed almost immediately after intrauterine injection, thus eliminating the possibility of a long-lasting effect within the reproductive tract.

The effect of the oestrous cycle stage on the uptake of ^{14}C -Glycerol and ^3H -Glucose from the mouse reproductive tract

The results of this phase of the experiment are presented in Fig. 2. These values were obtained from pooled data of all animals in any one stage of the oestrous cycle. The activity of ^{14}C -glycerol in the reproductive tracts of animals in oestrus was significantly greater than in metoestrous animals or dioestrous animals ($P < 0.05$), but did not vary significantly among animals which were in other stages of the oestrous cycle. The activity of ^3H -glucose in the reproductive tracts was significantly higher at oestrus than at any other stage of the oestrous cycle ($P < 0.01$), but did not vary significantly among animals in other stages. The accumulation of ^{14}C and ^3H in the liver was not significantly affected by the stage of the oestrous cycle. These results indicate that the trends for ^{14}C and ^3H were consistent in that the

relatively high activities in the reproductive tract were accompanied by relatively low activities in the liver for corresponding stages of the cycle. These results suggest that ^{14}C -glycerol and ^3H -glucose are not as rapidly absorbed from the reproductive tract at oestrus as at other stages of the oestrous cycle. The stage of the oestrous cycle has been shown to markedly influence the metabolic activity of the rat uterus (Nicollette and Gorski, 1964; Saldarini, 1967, 1968; Yochim and Saldarini, 1969; Yadava and Laumas, 1969). The results of the present study support the findings of Yochim and Saldarini (1969), who demonstrated that the rate of glucose incorporation by myometrial slices was variable during all stages of the oestrous cycle; the utilization was highest during dioestrus and lowest during metoestrus. They also noted that the percentage of utilized ^{14}C -glucose which appeared as labelled lactate increased between oestrus and dioestrus, being lowest at oestrus than at other phases of the oestrous cycle. The fact that less glucose and glycerol were absorbed from the reproductive tract during oestrus in the present study may be related to the general decline metabolic activity which occurs immediately after ovulation in rats (Kerly, 1937, 1940; Saldarini and Yochim, 1967).

The accumulation of ^{14}C and ^3H in the liver tissue as affected by the stage of the oestrous cycle, was consistent with the findings that a decrease in activity in the reproductive tract was accompanied by a corresponding increase in activity in the liver.

The physiological significance of these findings, namely that the highest activities of ^{14}C -glycerol and

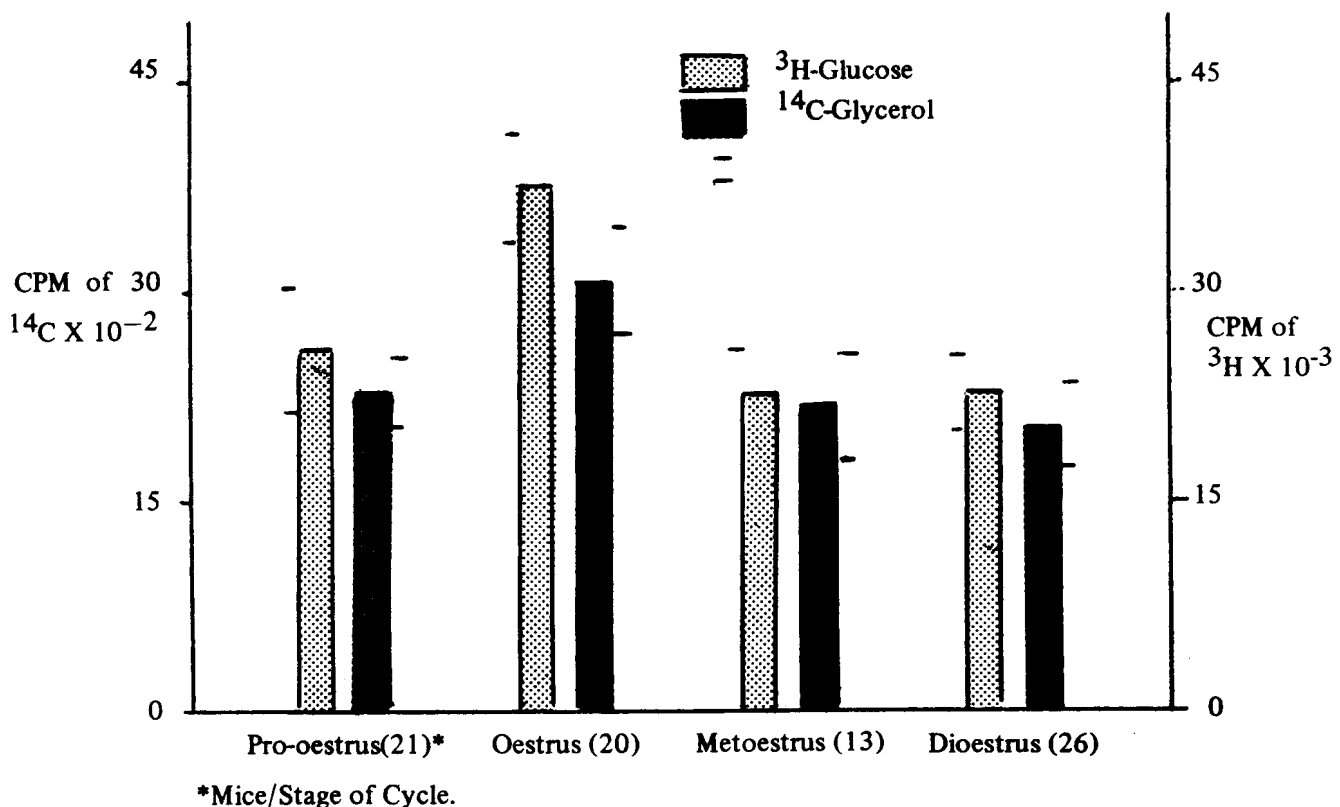


Fig. 2 The level of $1-^{14}\text{C}$ -Glycerol and $D-5-^3\text{H}$ -Glucose in mice reproductive tracts as affected by stage of the oestrous cycle

³H-glucose were in the uterus at oestrus, may be related to the apparent increase in epithelial cell mass and cellular activity of the endometrium at the time of oestrus (Brody and Wiqvist, 1961). An increase in the surface area of the endometrial tissue of the uterus at oestrus in addition to an increase in vascularity, could possibly enhance the absorptive

capacity of the uterus.

Further research is necessary to establish the significance of the rapid disappearance of inseminated compounds in the tract. Greater use should be made of isotopic tracers in studying spermatozoan transport and the final fate of spermatozoa within the female tract.

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