Zinc Transport in Rabbit Tissues

SOME HORMONAL ASPECTS OF THE TURNOVER OF ZINC IN FEMALE REPRODUCTIVE ORGANS, LIVER AND BODY FLUIDS

By J. E. A. McINTOSH and C. LUTWAK-MANN

*Agricultural Research Council Unit of Reproductive Physiology and Biochemistry,
University of Cambridge, Cambridge CB3 0JQ, U.K.*

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1. To investigate the influence of hormonal conditions upon the kinetics of zinc transport, specific radioactivity of ^{65}Zn was determined in certain tissues and fluids from unmated or pregnant rabbits during the first half of gestation. 2. Compartmental analysis was used to find the simplest mathematical model that simulated satisfactorily tracer behaviour. Models were fitted to experimental results by a numerical procedure using a computer. 3. The kinetics of zinc exchange in most tissues investigated could adequately be described by a three-compartment model, in which total tissue zinc content was divided into a rapidly exchanging pool, with a turnover time of about 1 h, and a slowly exchanging pool, the turnover time of which was in liver 15 h, in peak-stage corpus luteum 8 h, and in the other tissues 30–70 h. 4. In rabbit endometrium zinc transport varied with hormonal conditions, the turnover rate being higher in non-pregnant than pregnant endometrium. 5. Uptake of ^{65}Zn by uterine fluid was slow, and in the free-lying embryos (blastocysts) slower still, in keeping with uterine fluid acting as carrier of zinc into the unimplanted embryos. 6. In placental tissue zinc transport varied with gestational stage. Foetal placenta exchanged zinc with blood plasma four times faster than maternal placenta. In foetuses zinc turnover time and flux equalled that of the slow zinc compartment in foetal placenta. 7. Corpus luteum on days 5–6 of gestation showed peak specific radioactivity and zinc flux values, which exceeded those of all other tissues. 8. In liver the slow zinc compartment had a higher rate of turnover than corresponding compartments in tissues other than peak-stage corpus luteum, but no hormone-dependent changes were observed. 9. Zinc uptake by erythrocytes was the slowest of all examined.

Our interest in the metabolism of zinc stems partly from studies on endometrial carbonic anhydrase (McIntosh, 1970), the activity of which in the rabbit is controlled by progesterone (Lutwak-Mann, 1955; Lutwak-Mann & Adams, 1957), and partly from observations on the accumulation of zinc in the rabbit endometrium, which has been shown to coincide with the blastocyst phase of embryonic development (Lutwak-Mann & McIntosh, 1969). From other studies (Hurley & Swenerton, 1966) there are indications that zinc as a trace element is significantly involved in female reproductive performance, in that zinc deficiency in pregnancy leads to a high incidence of foetal malformation and mortality. Taken together, these findings suggest a hormonal influence on the metabolism and transport of zinc. In view of this, and as an extension of earlier work on the uptake of certain radioactive ions by the rabbit reproductive tract and early embryo (Lutwak-Mann *et al.*, 1960; Lutwak-Mann & McIntosh, 1971), we decided to study the transport of zinc in relation to the hormonal conditions that are operative respectively in oestrus and in the first half of pregnancy in the rabbit.

The approach used was to determine ^{65}Zn in tissues at various times after injection of this isotope and to calculate its specific radioactivity from measurements of the total zinc contents of the tissues. The results were then analysed by fitting mathematical models to the experimental results by using the method of compartmental analysis. This enabled us to estimate the sizes of the zinc compartments or pools and their turnover times, and the rates of zinc flux in the tissues examined.

Materials and Methods

Animals and tissues examined

Non-pregnant and pregnant rabbits from a cross-bred high-fertility colony were used; all animals were multiparous adults, mean weight 3.7 kg. The first half of pregnancy, extending from the day of mating (day 0) to day 14 (roughly mid-term), was investigated; in addition to the unmated and pregnant animals, pseudopregnant and superovulated rabbits were also used, the former to exclude the influence of the conceptus, the latter to observe the influence, if any, of

hormonal overstimulation. Eighty-seven rabbits were used in the experiments with ^{65}Zn , and 19 animals, not injected with ^{65}Zn but matched in respect of reproductive stage, for determining the total content of zinc in tissues. The following materials were examined (as available at a given reproductive stage): blood plasma, endometrium, early (undifferentiated) placental tissue, maternal and foetal placenta, foetus, ovarian interstitial tissue, corpus luteum and liver. Peritoneal fluid, oestrous uterine fluid, blastocyst fluid and foetal fluids were also investigated. Compartmental analysis was applied to the first group of tissues only. Tissues and fluids were recovered as described by Lutwak-Mann *et al.* (1960).

Injection of rabbits with ^{65}Zn and duration of experiments

$^{65}\text{ZnCl}_2$ (approx. 1Ci/g of zinc) in 0.1 M-HCl was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Each animal was injected subcutaneously with 10–15 μCi of ^{65}Zn (10–15 μg of zinc) in 1.8 ml of 0.01 M-sodium citrate buffer, pH 7. (Zinc ion was injected in the presence of a chelating agent, as otherwise precipitation occurred and movement of zinc from the site of injection was very slow.) Assuming that a 4 kg rabbit has 260 ml of blood (Dittmer, 1961), and that plasma contains 2 μg of zinc/ml (see below), the amount of zinc injected was a negligible proportion of the total plasma content and the steady state was therefore not disturbed. Tissues and fluids were sampled at 25 and 45 min (short-duration) and 4, 16 and 48 h (long-duration) intervals after injection of ^{65}Zn . About one-third of the rabbits were used to obtain results on the 45 min interval in the non-pregnant animals and on each day of pregnancy; the remaining two-thirds of the animals were used for the other time-intervals, but the experiments were limited to stages that are representative of clearly defined hormonal and developmental conditions of rabbit reproduction, namely: (i) non-pregnant (oestrus) and day of mating (both referred to as day 0); (ii) days 5, 6 and 7 (progestational phase, concurrent with the blastocyst stage); (iii) day 12 (post-implantation, concurrent with the foetal stage).

Determination of ^{65}Zn

Samples (100–800 mg) of tissue were weighed into capped plastic tubes and counted for radioactivity in a Wallac automatic gamma-ray spectrometer having a well-type NaI(Tl) crystal with an efficiency of 14%. Results were independent of tissue volume. At least 10000 counts were collected from every sample and, after background subtraction, the mean S.D. of duplicates was 4%.

Determination of zinc

Samples (50–150 mg) of tissue were weighed into semi-micro-Kjeldahl flasks and digested with 1 ml of fuming HNO_3 (analytical grade) and 1 drop of H_2O_2 . The residues were taken up in 4 ml of 0.01 M-HCl and zinc was determined with a Pye-Unicam SP.90 atomic-absorption spectrophotometer. Both internal and external standards were measured and a correction was applied for zinc in the HNO_3 . Glassware was soaked in 6 M-HCl for 24 h and rinsed well with deionized water before use. All determinations were in duplicate, with a mean S.D. of 5%.

Calculation of specific radioactivity

Measurements of radioactivity, expressed as d.p.m./g fresh wt. of tissue, were used to calculate uptake ratios, defined as (d.p.m./g)/(d.p.m. injected/mass of animal). The uptake ratio allowed a comparison to be made between the uptake of a tissue and the hypothetical uniform distribution of radioactivity throughout the animal. Small corrections were made to the uptake ratios for residual blood in the tissues, by using the values of plasma radioactivity. These corrections were only significant in the short-duration intervals, when the uptake ratio of the blood plasma was considerably higher than that of the other tissues. Each tissue after dissection was assumed to contain 1% of blood, except the liver, where 5% was allowed (Dittmer, 1961). Specific radioactivities were calculated in the form of ratios by dividing the uptake ratio for a particular tissue by the concentration of zinc in that tissue, which was itself expressed as a ratio.

Compartmental analysis

The analysis consisted of finding the simplest mathematical model that could satisfactorily simulate the observed kinetic behaviour of the tracer. A full description of the technique and its assumptions and limitations has been given (Solomon, 1960), including the fitting of functions by graphical means, and trial and error by using an analogue computer. Because these methods for fitting model functions to experimental data are imprecise and do not give an objective measure of the quality of the fit, they were replaced in the present investigation by a numerical procedure, with a computer program capable of fitting to data any function, not necessarily linear in its parameters, by the method of least squares. The program allowed for uncertainties in the experimental observations and gave an optimized value for each parameter in the fitting function, together with an estimate of its standard deviation and the value of reduced χ^2 for the fit. In addition, a graph of the points and a smooth curve describing the model were provided by a plotter connected to the computer.

Two simple models only were considered, details of which are given by Solomon (1960). The first was the two-compartment model illustrated schematically in Fig. 1(a), in which there is exchange between a reservoir of labelled zinc (the first compartment) and another tissue (the second compartment), initially containing unlabelled zinc only. The second model was the three-compartment situation illustrated in Fig. 1(b), in which there is exchange between the reservoir and two tissue compartments independently. This particular pattern of exchange is known as the parallel configuration; other patterns are possible (Solomon, 1960) but they are more complex mathematically. These alternatives may have offered better approximations to the real physiological situation than the parallel configuration, but to differentiate between them would have required very detailed experimental data. When the series version of the three-compartment model (Solomon, 1960) was applied to the results there were no improvements compared with the parallel model in the values of reduced χ^2 ; neither were the rate constants nor compartment sizes very different.

Blood plasma was assumed both to represent the reservoir of labelled zinc, and also to be the only path by which zinc is transported from one tissue of the body to another. In several cases it appeared from application of the criterion formulated by Zilversmit *et al.* (1943), which is discussed fully by Solomon (1960), that the two-compartment model was likely to yield a good description of the data. However, computer analysis indicated the better-fitting model and gave a measure of its suitability by calculating the

value of reduced χ^2 , a statistic equal to the variance of the data about the fitted function, divided by both the variance in the data itself and the number of degrees of freedom. The value of reduced χ^2 is expected to be approximately 1 when the model is a good description of the experimental observations, but as the value of reduced χ^2 increases, the probability that the model is a good one diminishes rapidly. For instance, when an attempt was made to fit the two-compartment model to the results for the foetal placenta, reduced χ^2 equalled 24, but on fitting the three-compartment model its value was diminished to 1.2, showing that the latter model is a satisfactory mathematical description of the data. How closely a model represents physiological reality can only be established by exhaustive experimentation under widely varying conditions. A suitable choice of either the two- or three-compartment model gave theoretical values that were in good agreement with the experimental results from all the tissues investigated. In most instances, however, values calculated for reduced χ^2 were higher than expected. This could be explained, at least in part, by the fact that when the parameters of the blood plasma-fitting function were introduced into the models of the tissues, there was no convenient way of incorporating the uncertainties in each parameter. The omission of these uncertainties was equivalent to underestimating the variance in the tissue data, which in turn led to exaggerated values of reduced χ^2 .

Compartmental analysis was done in the following way. First, a mathematical description was found for the disappearance of tracer from the blood plasma reservoir. The parameters of this function served to transfer information about the way in which the value for specific radioactivity in the blood plasma was changing to the two- and three-compartment models fitted to the results for the other tissues. The model function describing the two-compartment system contains a single variable parameter equal to the rate constant for the transport of zinc out of the tissue compartment; it is the fraction of the zinc in the tissue compartment transported to the blood plasma in unit time. The function describing the three-compartment model contains three parameters to be optimized: two rate constants (one for each of the two tissue compartments) and the size of one of the compartments expressed as a fraction of the whole; the size of the other can be found by difference. Because there is no experimental access to the individual pools of zinc but only to their mean specific radioactivity, the fitting function is expressed as a sum of the individual specific radioactivities.

Turnover time, or the time taken for an amount of zinc equal to the total contained in a tissue compartment to be transported across its boundary to the reservoir, is equal to the reciprocal of the rate constant. The flux of zinc from a tissue compartment to

Fig. 1. Block diagrams representing the two- and three-compartment models described by the mathematical functions

(a) Open two-compartment system, to which the Zilversmit *et al.* (1943) criterion can be applied if two conditions are met: (i), that the system be in the steady state, and (ii), that there be no other route of entrance to the tissue compartment except through the plasma compartment. (b) Open three-compartment system, showing the parallel version of the general model.

the reservoir, which equals the product of the rate constant and the zinc content of the compartment, must equal the zinc flux in the opposite direction when the system is in the steady state.

The driving force for tracer movement in a system in the steady state is the specific radioactivity of the tracer within the compartment from which the flux originates; in a closed system, at infinite time after injection of tracer, there must be equal specific radioactivity in every compartment. The analytical complications that arise in experiments with rapidly growing tissues (such as the foetus), where a steady state is not maintained, will be referred to below.

Results

Zinc concentration in the tissues

It can be seen from Table 1 that the liver was the tissue richest in zinc. Apart from progestational endometrium (days 5–7), which, in confirmation of our earlier report (Lutwak-Mann & McIntosh, 1969), had twice the zinc concentration of the non-pregnant oestrous tissue, the concentrations of zinc in all other tissues examined did not differ significantly with the stage of pregnancy. The values for placental tissue, ovarian luteal and interstitial tissue, and foetus, were

Table 1. *Concentration of zinc in rabbit tissues*

The numbers in parentheses after the names of the tissues denote the days of gestation on which the tissues were recovered (day of mating is referred to as day 0); where there were no significant differences, the values were pooled for each period shown in parentheses. When not indicated specifically, materials were collected throughout the entire period. The results are expressed as means $\pm$ S.E.M. with the S.D. in parentheses.

Tissue	Concn. of Zn ($\mu\text{g/g}$ fresh wt.)
Blood plasma	2.02 ± 0.13 (0.82)
Endometrium (non-pregnant)	9.60 ± 0.30 (1.5)
Endometrium (5–7)	17.2 ± 0.51 (2.8)
Undifferentiated placental tissue (7–9)	13.0 ± 0.83 (2.0)
Maternal placenta (10–14)	9.96 ± 0.34 (1.1)
Foetal placenta (10–14)	12.0 ± 0.60 (1.8)
Foetus (12–14)	8.03 ± 0.43 (1.5)
Corpus luteum (3–14)	11.5 ± 0.32 (1.6)
Interstitial ovarian tissue (0–14)	12.8 ± 0.20 (1.5)
Liver	33.5 ± 0.84 (5.9)
Peritoneal fluid	0.70 ± 0.14 (0.71)
Uterine fluid (0)	2.06 ± 0.35 (0.98)
Blastocysts (6)	1.60 ± 0.19 (0.63)
Amniotic fluid (10–14)	1.02 ± 0.15 (0.43)

all within the range 8–13 μg of zinc/g fresh wt. No differences in zinc content were detected in progestational endometrium, or luteal or interstitial ovarian tissues, when these were taken from superovulated or pseudopregnant animals. Peritoneal fluid, oestrous uterine fluid, blastocyst fluid and the foetal fluids (exocoelomic, allantoic and amniotic fluids) gave very low values. Individual variability was marked in all these fluids; contamination was ruled out as the cause.

Specific radioactivity of ^{65}Zn in blood plasma and the function fitted to the experimental values

The results of measurements on samples of blood plasma (Fig. 2a) from 70 rabbits were fitted satisfactorily by a function consisting of the sum of two exponential terms. Although the injection of tracer was given subcutaneously and not intravenously, the passage of radioactivity into the blood stream was very rapid, as can be seen from the fact that the amount of tracer in the blood plasma was already falling by 25 min after injection. Details of the model function and its parameters are given in Table 2.

Compartmental analysis

Fig. 2 presents the experimental results to which compartmental analysis was applied, expressed as specific activity in each tissue against time after injection of ^{65}Zn ; also shown are the curves representing the fitted models. Results of fitting models to the experimental results are given in Table 3.

Table 2. *Parameters in the function fitted to the values of specific radioactivity of ^{65}Zn in blood plasma in relation to the time-interval after injection of label*

The function $p_1^* = A_1 \exp(-A_2 t) + A_3 \exp(-A_4 t) + A_5$ was fitted to the pooled specific radioactivities in blood plasma from 70 animals at all experimental stages. p_1^* equals the specific radioactivity at time t after injection. The function consists of a term describing a fast process of zinc exchange (A_1 and A_2), a term describing a slow process (A_3 and A_4) and a constant (A_5) representing the equilibrium asymptote. When fitting models to results for the other tissues it is necessary to relate these to the decrease with time in the specific radioactivity of the blood plasma; only the first four parameters are required for this procedure.

	A_1	A_2	A_3	A_4	A_5
Parameter	1.7×10^4	0.627	243	0.010	32
S.D.	0.12×10^4	0.079	92	0.006	96
Reduced χ^2	= 0.51

Fig. 2. Results of experiments with rabbit tissues to which compartmental analysis was applied, expressed as variation in specific radioactivity of ^{65}Zn in relation to time after injection of tracer

The curves represent the fitted models. Where the reproductive conditions are not given, the tissues were collected throughout the period examined (days 0–14 of gestation). ●, Blood plasma. (a) △, Non-pregnant endometrium; ○, 5- to 7-day pregnant endometrium. (b) △, 12-day foetal placenta; □, 12-day maternal placenta; ○, 12-day foetus. (c) △, 5- to 6-day luteal tissue; □, 8- to 14-day luteal tissue; ○, interstitial ovarian tissue. (d) △, Liver; ○, erythrocytes. The uncertainties, represented as vertical bars, are equal to S.E.M.

Non-pregnant and pregnant endometrium. In agreement with the rise in zinc concentration known to occur in the progestational endometrium, there was almost a doubling in ^{65}Zn uptake by this tissue in

long-term experiments. It is clear from the experimental results for the non-pregnant and progestational endometrium (Fig. 2a), that, in rabbit endometrium, the kinetics of zinc transport were distinctly modified by the hormonal state of the animal.

Compartmental analysis showed that a two-compartment model was adequate for the non-pregnant endometrium but that the three-compartment model provided a better description of the behaviour of the pregnant endometrium. The turnover time of the single zinc compartment in the non-pregnant tissue (Table 3) was shorter than that of the major (94%) pool in the pregnant endometrium. The fluxes of zinc at the two different hormonal stages were the same because the pregnant tissue had twice the concentration of zinc.

Maternal and foetal placenta. The specific radioactivities of both placental tissues were found to vary with the progress of gestation. Fig. 3 illustrates the situation 45 min after administration of ^{65}Zn , in the maternal and foetal placenta (days 10–14 of pregnancy); in addition, results are included for the specific radioactivity of endometrium (days 0–10) and early, not yet fully differentiated, placental tissue (days 7–9). The high specific radioactivity in the oestrous endometrium fell steeply as early pregnancy progressed. Values for progestational endometrium and undifferentiated placental tissue were almost equal; by day 10, a sharp and sustained rise in values for foetal placenta became evident whereas those for maternal placenta declined slightly. This means that the rate of transport of zinc between the foetal placenta and the blood plasma increased as pregnancy advanced, whereas in the maternal placenta it decreased.

Compartmental analysis was applied to the placental tissues collected on day 12 of pregnancy (Fig. 2b and Table 3), that is, at a point at which this divergence was already pronounced. In terms of the analytical model used, the maternal placenta contained a small (3%) pool of rapidly exchanging zinc, but in the foetal tissue 28% of the zinc exchanged rapidly with the blood plasma. This difference in compartment size explains the disparate results for the two tissues in the short-duration interval illustrated in Fig. 3. Comparison of the total rates of zinc flux in the two tissues (Table 3) shows that the foetal placenta exchanged zinc with the blood plasma at almost four times the rate of the maternal placenta.

Foetus. The results with 12-day-old foetuses are given in Fig. 2(b). The investigation was confined to a single day of foetal development because, owing to the fast foetal growth in this phase, the foetus cannot be considered to be in a steady state for more than a limited period of time. Compartmental analysis (Table 3) indicated that the turnover time of the foetus was like that of the slow compartment in the foetal placenta, and that the foetus lacked a faster

Table 3. Results of fitting the models to the experimental results from each tissue, expressed in terms of compartment size, turnover time and flux of zinc

Turnover time is the time taken for an amount of zinc equal to the total zinc in a compartment to pass in one direction across its boundary. For the three-compartment model, the tissue compartment exchanging zinc more rapidly is listed first. Numbers in parentheses after the names of tissues indicate the day of gestation. Numbers in parentheses in the last column are the values of reduced χ^2 for the alternative, but unsuitable, model. Results for the ovarian tissues, reported previously (McIntosh & Lutwak-Mann, 1971), are not included. Uncertainties are expressed as s.d.

Tissue	Compartment size (% of tissue Zn content)	Turnover time (h)	Flux	Type of model (and reduced χ^2)
			(μg of Zn transported to or from compartment/h per g fresh wt. of tissue)	
Non-pregnant endometrium	100	8.1 ± 0.4	1.2 ± 0.06	Two-compartment 2.5 (3.1)
Pregnant endometrium (5-7)	6 ± 3 94 ± 3	1.5 ± 0.6 34 ± 4	0.74 ± 0.40 0.47 ± 0.06	Three-compartment 3.7 (19)
Maternal placenta (12)	3 ± 2 97 ± 2	0.6 ± 0.6 70 ± 12	0.44 ± 0.50 0.14 ± 0.02	Three-compartment 1.6 (11)
Foetal placenta (12)	28 ± 8 72 ± 8	1.7 ± 0.6 47 ± 19	1.9 ± 0.9 0.19 ± 0.09	Three-compartment 1.2 (24)
Foetus (12)	100	45 ± 7	0.18 ± 0.03	Two-compartment 0.42
Erythrocytes	100	121 ± 10	0.066 ± 0.010	Two-compartment
Liver	5 ± 1 95 ± 1	1.1 ± 0.2 15 ± 1	1.4 ± 0.5 2.2 ± 0.1	Three-compartment 5.9 (13)

compartment, which, in the foetal placenta, involved 28% of the total zinc. The fluxes of zinc in the foetus and the slow compartment of the foetal placenta were identical.

Corpus luteum and ovarian interstitial tissue. In a preliminary note (McIntosh & Lutwak-Mann, 1971) we have compared specific radioactivities in the corpus luteum and interstitial ovarian tissue at one point only, namely 45 min after injection of ^{65}Zn . We found that on days 5 and 6 of pregnancy the specific radioactivity in luteal tissue rose to a sharp peak; in the interstitial tissue there was a simultaneous, but only minor, upward trend. Compartmental analysis, with a three-compartment model, indicated that the peak in luteal zinc exchange was explained by a seven-fold increase in zinc flux in the slower-exchanging luteal compartment, caused by a decrease in turnover time.

Fig. 2(c) records results for both the short- and long-duration experiments, separately for luteal tissue at the peak stage (days 5 and 6) and outside the peak (days 8-14) as well as ovarian interstitial tissue. The specific radioactivity at the luteal peak stage can be seen to exceed all other tissues examined. Comparison of total zinc flux in the peak-stage corpus luteum (McIntosh & Lutwak-Mann, 1971) with other tissues having elevated rates of flux (Table 3) showed it to be the highest, the amount of zinc transported to or from

each g of luteal tissue in 1 h being $4.6 \mu\text{g}$, against, for example, $3.6 \mu\text{g}$ for liver and $2.1 \mu\text{g}$ for foetal placenta.

Liver. Inspection of Fig. 2(d) might suggest that a two-compartment model would have described the data for this tissue but, in fact, the three-compartment model was distinctly superior. It can be seen from Table 3 that the slowly exchanging compartment of the liver showed a much higher rate of zinc turnover than the corresponding compartments in other tissues. Because of the elevated zinc content, the total flux of zinc in this organ was high.

Erythrocytes. The specific radioactivity of ^{65}Zn in erythrocytes was obtained by difference from measurements made on whole blood and blood plasma, two rabbits being used. A value of 37% was assumed for the packed cell volume (Dittmer, 1961) and the zinc concentration in erythrocytes was taken as $9 \mu\text{g/g}$ (Berfenstam, 1952). It can be seen (Fig. 2d) that the rate of movement of ^{65}Zn into erythrocytes was slow. The two-compartment model fitted the data adequately and a value of 120 h for turnover time was obtained (Table 3); the validity of applying this type of analysis to zinc uptake by erythrocytes is discussed below.

Peritoneal fluid, blastocyst fluid, uterine fluid and foetal fluids. Partly because of their low zinc contents and partly owing to considerable individual variability, the results of measurements on these

Fig. 3. Specific radioactivity of ^{65}Zn in tissues at 45 min after injection of label

Measurements were made in rabbit endometrium (Δ), early undifferentiated placental tissue ($\blacktriangle$) and foetal ($\bullet$) and maternal ($\circ$) placenta, during days 0–14 of pregnancy. The uncertainties, represented as vertical bars, are equal to S.E.M.

materials were not suitable for compartmental analysis. The values below are expressed both as uptake ratios and specific radioactivities ($\times 10^{-5}$), at points when these parameters were maximal and also at the termination of our experimental time-scale (48 h); the corresponding values for blood plasma are given in parentheses. Peritoneal fluid had a maximum value of uptake ratio at 4 h, 0.3 (0.7), specific radioactivity 4.3 (3.4); at 48 h, 1.4 (1.2). Blastocyst fluid had low values, which rose slowly so that the maximum uptake ratio was reached only at 48 h, 0.17 (0.25), specific radioactivity 1.0 (1.2). In uterine fluid the maximum value of uptake ratio was at 16 h, 0.24 (0.35), specific radioactivity 1.2 (1.8); at 48 h, 1.1 (1.2). Of the foetal fluids only amniotic fluid was available in sufficient amounts; the maximum value for uptake ratio was at 48 h, 0.08 (0.25), specific radioactivity 1.1 (1.2).

Discussion

The experiments on zinc transport and exchange were done parallel with a study of the transport of calcium (Lutwak-Mann & McIntosh, 1971), which meant injecting the same animals simultaneously with both ^{65}Zn and ^{45}Ca . This makes the results obtained with these two isotopes directly comparable and it is useful to consider together some

instances of the behaviour of zinc and calcium in the different experimental situations examined.

Concerning first the influence of hormonal conditions operative at the reproductive stages investigated, it can be seen that, for example in the rabbit endometrium, the rate of zinc turnover was markedly higher in the tissue from non-pregnant than pregnant animals. An analogous difference in this respect was also observed in the rate of calcium exchange.

When discussing results obtained with rabbit blastocysts, one must remember that, as they lie free in the uterine lumen during days 5–7 of gestation, these embryos possess no blood links with the maternal organism and their sole source of nutrient supply is the secretion produced by the progesterational endometrium. In this respect it is noteworthy that whereas the concentration of zinc in the blastocysts was very low in relation to the surrounding zinc-rich endometrium, the calcium concentration gradient between blastocysts and uterine mucosa was small.

The results on zinc transport in blastocysts indicated that the rate of uptake of ^{65}Zn by the embryos was slow in comparison with the rate of isotope uptake from the maternal blood plasma by the supporting endometrium. The situation was similar in experiments with calcium, but the uptake of ^{45}Ca by the blastocysts was appreciably faster than that of ^{65}Zn .

Although for reasons referred to above we were unable to apply compartmental analysis to the data recorded for blastocysts and uterine fluid, we observed that the uptake of maternally transmitted ^{65}Zn by the uterine fluid (in comparison, for example, with peritoneal fluid) was slow, and in the blastocysts slower still. This is compatible with the uterine fluid acting as the chief vehicle for the transfer of nutrient, in this case zinc, into the blastocyst compartment. Although the rate and extent of the zinc exchange in these systems were low, it is clear that zinc transport is functional already at this early stage of embryonic development.

As to the more advanced, post-implantation stage of pregnancy, when comparing the placental tissues and the foetus one finds that whereas the foetal placenta had a high zinc flux per unit weight of tissue, the corresponding value for the foetus amounted to only 10% thereof. The rate for the foetus was calculated by assuming the existence of direct exchange between it and the maternal blood plasma, as was done for all the other tissues; this approach gave good agreement with the experimental data for the foetus. The evidence of active zinc metabolism in the foetal placenta, in relation to the fast-growing foetus, fits in satisfactorily with the well-established role of zinc as an essential trace element in animal reproduction.

In the discussion of exchange processes reported here attention should be drawn to one more point,

namely that the term 'exchange' is probably not strictly applicable to an experimental object such as the young foetus in which, because of the high rate of growth, the total zinc content rises concomitantly. Therefore a steady state is not maintained, and the investigation of the kinetics of transport becomes rather complex. It is for this reason that a single day only (day 12 of gestation) was selected for study; it was regrettable that our 48 h experimental interval exceeded this 1-day limit. A similar argument, though to a lesser degree, also applies to tissues such as the maternal and foetal placenta.

Another aspect worth commenting upon is that although the higher flux of zinc in the foetal than in the maternal placenta resulted from the existence of a substantially larger pool of rapidly exchanging zinc in the foetal organ, it was shown, with the same type of model, that in the luteal tissue of the rabbit ovary the sevenfold rise in zinc flux, characteristic of days 5–6 of gestation (McIntosh & Lutwak-Mann, 1971), was caused by a decrease in the turnover time of the slower-exchanging compartment, without any accompanying change in the sizes of the compartments involved.

The liver was included in the investigation because it is a storage organ for zinc. During the first half of pregnancy in the rabbit there was no discernible hormone-dependent change in the zinc concentration of liver. Similarly, there was no significant variation in the amount of uptake of ^{65}Zn by the liver at 45 min after injection of the isotope, indicating that, like its zinc content, the flux of zinc to this organ remained constant.

The movement of ^{65}Zn from the maternal blood plasma into the erythrocytes was slow in comparison with the other tissues. This long turnover time may represent an exchange of zinc between the erythrocytes and the blood plasma, but another explanation is more likely. Nearly all zinc in erythrocytes is known to be incorporated into carbonic anhydrase, and this zinc cannot be removed from the enzyme under physiological conditions (Lindskog & Malmström, 1962). It is therefore conceivable that the passage of ^{65}Zn into erythrocytes occurs during the synthesis of carbonic anhydrase, which takes place in the bone

marrow. This being so, compartmental analysis, as applied above, would not be valid because under such circumstances a steady state does not exist.

The kinetics of zinc exchange in the majority of the tissues that we have investigated were adequately described by a three-compartment model, in which the total zinc content of the tissue was divided into a rapidly exchanging pool, with a turnover time of about 1 h, and a slowly exchanging pool, the turnover time of which in the liver was 15 h, in the peak-stage corpus luteum 8 h, and in the other tissues 30–70 h. At the present time there is no evidence to allow a firm association to be made between these pools of zinc and any known biochemical processes; provisionally one might envisage that the rapid zinc exchange is concerned with its movement into either intra- or extra-cellular fluid, whereas the slower rate of exchange may be linked with long-lasting events in which zinc is incorporated into, for instance, zinc-containing enzymes.

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