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Penetration of Chemicals into the Oocyte, Uterine Fluid, and Preimplantation Blastocyst

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Chemicals, including commonly used drugs (e.g., penicillin, meprobamate, pyridium, and mercaptomerin) penetrate and persist for some time in the ovarian follicular fluid at concentrations approximately similar to that of the serum. Information as to the penetration of chemicals into the granulosa cells and into the oocyte is scanty, although there are some indications that these structures are also permeable to foreign chemicals. Similarly, caffeine, nicotine, thiopental, salicylic acid, antipyrine, barbital, and isoniazid enter the uterine secretion and penetrate the preimplantation blastocyst of mice, rats and rabbits. The pattern of distribution of compounds among ovarian follicular fluid, uterine luminal fluid, blastocyst and plasma varies from compound to compound and appears to be related to the molecular weight and degree of ionization of the compound and differs in pregnant and nonpregnant animals. Thus, nicotine and DDT accumulate in the uterine luminal fluid of pregnant but not in that of nonpregnant rabbits.

The penetration of foreign chemicals into the oocyte, uterine luminal fluid, and preimplantation blastocyst may exert adverse effects on fertilization, implantation, and/or further development of the conceptus. The possible toxicological importance of this process to eutherian reproduction is discussed.

It has been well established that many chemicals, including drugs, are transferred from the maternal body compartments to the conceptus during its intrauterine life. However, it is apparent from the literature that most of the information available is related to the passage of drugs across the placenta in late pregnancy. Much less is known about the transfer of compounds across the early placentation when the conceptus, at the embryonic stage, is sensitive to the teratogenic action of chemicals (*1–3*).

It has become increasingly evident that drugs given to the mother can also affect the development of the conceptus during the stages of pregnancy which precede implantation. Drugs and other chemicals administered to the mother are known to prevent implantation (*4*), cause degenerative changes in the blastocyst (*5*), alter the blastocyst protein profile (*6*), or adversely affect the development of the free blastocyst (*7*). There is also some concern that reproduction may be affected by environmental agents before fertilization, since foreign chemicals not only are readily transferred into the gonads, but they may persist in these organs for relatively long periods after exposure. For example, following a single dose of DDT given to pregnant New Zealand rabbits, DDT and some of its metabolites penetrated the maternal ovaries and persisted there for at least 17 days after exposure. Even at that time, the concentration of DDT in the ovaries was almost 80 times higher than in the plasma (*8*).

The maturing ovarian follicle is an avascular structure, regardless of follicular size or state of follicular maturation (*9, 10*). Therefore, every molecule destined to enter the follicle must leave the vascular bed of the perifollicular region and cross the thecal layer before entering the follicular fluid and/or the granulosa cells. To reach the oocyte, molecules may either be transported by the granulosa cells or they may advance passively in follicular fluid through the intercellular spaces and/or the antrum.

At the beginning of the century, it was found that occlusion of the ovarian vein causes a doubling of follicular size and often rupture of the follicle in a matter of seconds (*11*). This observation suggested

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that a high degree of permeability existed in the barrier between blood and follicular fluid. Early experiments, showing that Evans blue, S^{35}, and P^{32} were found in the follicular fluid soon after parenteral administration (*12, 13*) confirmed this hypothesis. In 1958 it was shown that tritiated water (*14*) and some commonly used drugs including meprobamate, penicillin, pyridium, and mercaptomerin rapidly equilibrated between blood and follicular fluid when administered orally or parenterally to women (*15*). High molecular weight compounds (e.g., I^{131} γ-globulin) also penetrate the follicular fluid (*16*), and recently it has been shown that the blood–follicle barrier behaves like a molecular sieve, allowing passage of proteins in inverse proportion to their molecular weight (*17*).

Information as to the penetration of chemicals into the granulosa cells and into the oocyte is scanty, although there is some evidence that these structures are also permeable to foreign chemicals (*18–20*). Moreover, Glass (*16*) has shown that albumin and α-globulin penetrate the oocyte, but that β- and γ-globulins do not.

These results, although incomplete, certainly suggest that all the components of the ovarian follicles are readily permeable to endogenous as well as exogenous chemicals. It is likely that a process of simple diffusion, at least for the small molecular weight foreign compounds, is involved. However, the importance of physicochemical characteristics (i.e., lipid solubility and degree of ionization) in determining the rate and degree of entry has still to be clarified.

A few reports have described the passage of chemicals into the oviduct and into the conceptus during the premorula stages of pregnancy (*21, 22*); relatively more information is available on this process after the conceptus has entered the uterine cavity and become a blastocyst.

Some of the first indications that substances can pass from the maternal circulation into the preimplantation blastocyst derived from observations by Greenwald and Everett (*23*) and Lutwak-Mann, Bournsnell, and Bennett (*24*). The former workers demonstrated the uptake and incorporation of ^{35}S-methionine by preimplantation blastocysts of mice treated parenterally with this compound. Lutwak-Mann's group showed passage of $^{32}PO_4$, ^{42}K, $^{35}SO_4$, ^{24}Na, and ^{131}I into endometrial fluids and preimplantation blastocysts of rabbits receiving these ions parenterally.

In our laboratory, we have shown that, following the oral or parenteral administration of radioactive caffeine, nicotine, salicylate, isoniazid, antipyrine, barbital, thiopental, and DDT, significant amounts of the unchanged compounds as well as some of their metabolites are present in the uterine fluid of rabbits, rats, and mice (*25, 26*). Most of the small molecular weight chemicals attained concentrations in the uterine fluid similar to those of the plasma at the corresponding times. Larger molecular weight compounds (i.e., insulin and dextrans) did not pass into the uterine fluid readily. It would appear that passage of foreign chemicals into the uterine fluid is influenced by the molecular weight of the compounds, as well as by their degree of ionization at physiological pH (*27*). Similar results were obtained by Conner and Miller (*28*), who studied the passage of ^3H_2O, barbital, tetraethylammonium bromide, α-aminoisobutyric acid, tetraethylammonium dimethyloxazolidinedione, ouabain, insulin, and antipyrine into the uterine luminal fluid of the rat. They showed that the distribution of compounds into luminal fluids correlated in a general way with lipid solubility at pH 7.4 and that no special transport systems were involved in the transfer of the substances studied.

Foreign chemicals pass into the secretion of several exocrine glands (e.g., saliva, bile, sweat, tears, milk, and seminal fluid) and this transfer is dependent upon the pH of the secretion (*29, 30*), the molecular weight (*31*), and the degree of ionization of the compound (*32, 33*). It was not unexpected, therefore, that these factors can also influence the passage of chemicals into the uterine fluid which is primarily a product of exocrine glands.

One of the most intriguing observations we made in these studies was that pregnancy may modify the degree to which drugs pass into the uterine fluid. For most of the compounds examined the degree of transfer into the uterine fluid was not different in nonpregnant and 6-day pregnant rabbits (*34*). However, this was not the case for nicotine and DDT, which accumulate in the uterine luminal fluid of 6-day pregnant rabbits but not in that of nonpregnant does similarly treated (*35*). For example, 30 min after intravenous treatment of 6-day pregnant rabbits with ^3H-nicotine (50 μg/kg), 36-fold higher concentrations of unchanged nicotine were found in uterine fluid than in plasma. Cotinine, a major metabolite of nicotine, also accumulated in uterine fluid, but to a lesser extent. In contrast, the concentration of unchanged nicotine in uterine fluid approximated that in plasma of similarly treated nonpregnant rabbits (*35*).

The mechanism by which nicotine accumulates in the uterine fluid of pregnant rabbits has intrigued us for some time. We first attempted to determine whether a pH gradient between the uterine fluid and plasma of 6-day pregnant rabbits could account for the accumulation of nicotine in the uterine fluid, since this mechanism has been used to explain the

accumulation of ephedrine in the milk (*33*) and quinine in the gastric fluid (*29*). However, the pH of uterine fluid of 6-day pregnant animals (pH 7.68) is not significantly different from that of nonpregnant animals (pH 7.61) (*36*), ruling out this postulated mechanism for the accumulation of nicotine in the pregnant uterine fluid. These findings are in agreement with those of others who have reported accumulation of foreign chemicals in the secretion of exocrine glands which cannot be explained by a pH gradient. For example, the milk/plasma concentration ratio for quinine and erythromycin has been reported to be 4.8 and 8.7, respectively (*33, 37*); urea reaches levels in human sweat two to four times that of plasma (*38*) despite the absence of pH gradients between the compartments.

We were also unable to demonstrate any difference in the physiological disposition of nicotine between 6-day pregnant and nonpregnant does which could account for the accumulation of nicotine in the pregnant uterine fluid. Thus, the volume of distribution, plasma half-life, rate of metabolism, urinary excretion, and plasma protein binding of nicotine are similar in pregnant and nonpregnant animals (*35*).

These findings indicate that the endometrial tissue in the functional stage of pregnancy influences the active transfer of nicotine into the uterine fluid. This hypothesis is supported by the fact that the accumulation of nicotine seen in the 6-day pregnant rabbit can be reproduced by pretreating nonpregnant rabbits with either human chorionic gonadotrophin or progesterone (*35*).

Parenterally and orally administered drugs and other chemicals not only enter the endometrial fluid but also penetrate the free-lying blastocyst. Sodium thiocyanide, sulfonamide, and salicylate (*39, 40*), thalidomide (*41–43*), as well as DDT, isoniazid, barbital and thiopental, caffeine and nicotine (*26, 34*) have been identified in blastocysts of the rabbit following maternal treatment. Experiments *in vitro* showed that dextran of 60,000 to 90,000 molecular weight does not penetrate the 6-day rabbit blastocyst, whereas 12 other compounds of smaller molecular weight, including 16,000 to 19,000 molecular weight dextran, salicylate, sulfanilamide, antipyrine, and hexamethonium, enter the blastocyst at a rate which seems dependent upon their lipid solubility and degree of ionization.

Although it is clear from these findings that the preimplantation blastocyst is permeable to numerous foreign compounds, additional studies are necessary to clarify the mechanisms by which these substances enter the free-lying blastocyst and distribute themselves.

It is of interest in this respect that when 6-day preimplantation rabbit blastocysts were incubated for 60 min in a medium containing ^{14}C-DDT, the embryonic disc with its associated zona-coated trophoblast contained a concentration of DDT which was approximately fivefold that of the blastocoelic fluid, which by this time had equilibrated with the medium (*35*). In view of these results, it would be interesting to know whether compounds such as 6-mercaptopurine, actinomycin D, and thalidomide, which have been shown to cause abnormalities of the embryonic disc and trophoblast (*44*) preferentially accumulate in these tissues.

The presence of a number of foreign chemicals in the preimplantation blastocyst following maternal exposure has raised the obvious question of the toxicological significance of these findings.

In our laboratory, we have administered daily doses of caffeine (25 mg/kg, orally), salicylate (100 mg/kg, orally), DDT (1 mg/kg, orally), and nicotine (0.1 mg/kg, intravenously) to pregnant New Zealand White rabbits on days 4 through 7 of gestation and evaluated the outcome of pregnancy. Neither caffeine nor salicylate, administered to rabbits during the preimplantation stages of pregnancy, appeared to exert any toxic effects on the conceptus. Treatment with nicotine did not reduce the number of implantations or the volume of the conceptus at 8 days, although the average weight of the offspring recovered at 28 days of pregnancy was significantly ($p < 0.05$) lower than control fetuses. It has been shown that women who smoke have a higher spontaneous abortion rate (*45*), and the babies they deliver are more likely to be premature (*46*) and have relatively lower birth weights (*47*). Whether this type of human toxicity has any relationship to exposure to nicotine during the preimplantation stages of pregnancy remains to be assessed.

Treatment with DDT during the preimplantation stages of pregnancy did not decrease the number of implantations, but the average volume of the implanted embryos at 8 days of pregnancy was significantly less than that of the controls. As in the case of nicotine, the 28-day fetuses of the DDT-treated rabbits were significantly smaller than those of the control group. In addition, brain weights of the DDT-exposed fetuses were significantly lower than the brain weights of the control animals. These effects were not seen with caffeine, salicylate, or nicotine. Since Wigglesworth (*48*) and Harding and Shelley (*49*) have reported that during intrauterine life the increasing brain weight of rat and rabbit fetuses parallels gestational age and is relatively independent of the total body weight of the conceptus, the decrease in fetal brain weight of DDT-treated animals may be of significance.

This may well be an indication that, at least in the

rabbit, DDT exposure during the preimplantation stages of pregnancy exerts a toxic action which is manifested by intrauterine growth retardation. It has been reported that DDT causes prematurity and intrauterine growth retardation when given in large amounts to pregnant rabbits during morphogenesis (*50*), and low birth weights in human infants have been correlated with high serum levels of DDE, a major metabolite of DDT (*51*). Thus, our results in the rabbit seem to indicate that exposure of the mother during the preimplantation stages of pregnancy to some commonly encountered environmental agents such as nicotine and DDT may result in a type of toxicity manifested by intrauterine growth retardation in the absence of teratogenesis (*27*).

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