to increase the antibody content only by a factor of 2 in the males fed laboratory chow and approximately 5 in the females. This indicates that the adjuvant effect of diet 3 on the response of the immune system to SRBC is considerably greater in the males but not the females than that obtained with LPS. While the combination of diet 3 and LPS did not significantly affect antibody levels in the males, it had an adjuvant effect (by a factor of 4) in the females. This shows that a complementary action of diet 3 and LPS is found in the females only. Notwithstanding these sex-related differences, both males and females showed similar minimum and maximum antibody levels. These findings may be interpreted in terms of a sex-related stimulation of the hematopoietic system by diet 3. In the males, a greater uptake of ^{3}H-TdR by the hematopoietic tissues has already been shown when animals were fed the elemental diet instead of laboratory chow[4,7]. In the females, on the other hand, a significantly lower uptake of ^{3}H-TdR by the spleen and the lymph nodes was obtained under the same nutritional conditions[2]. It appears then that diet 3 affects the cellular systems responsible for the immune response to a different extent in the males and the females. Such a dietary effect would also account for the sex-related differences observed in the adjuvant action of LPS. An alternative interpretation that antibody production or turnover is modified by the elemental diet is presently under investigation.

The influence of formula-defined diets on the response of the immune system is likely to have clinical importance. Such diets are already fed tumour-bearing patients to assure proper nutritional and systemic balance during cancerotherapy[8–10]. Of particular interest is our finding that diet 3 affects anti-SRBC antibody production to a similar and even greater extent than LPS. Lipopolysaccharides have been and are still investigated as promising adjuvants in the treatment of cancer[11].

Table 2. Effects of diet 3, sex and LPS mR 595 on the response of the immune system to SRBC in the rat

Anti-SRBC antibody levels determined in serum by passive hemagglutination				
Sex	Animals fed laboratory chow		Animals fed diet 3	
	SRBC[a]	SRBC-LPS[b]	SRBC	SRBC-LPS
Males	3.50 ± 0.85[c]	4.67 ± 0.61	8.00 ± 0.32	7.00 ± 0.45
	11[d]	25	256	128
	(6–20)[e]	(17–39)	(205–320)	(94–175)
Females	3.61 ± 0.51	5.83 ± 0.38	6.45 ± 0.37	8.50 ± 0.22
	12	57	87	362
	(9–17)	(44–74)	(68–113)	(311–422)

[a] SRBC: sheep erythrocytes (1 ml, 10^8 cells) injected i.p. [b] LPS: 40μg injected i.p. simultaneously to SRBC. [c] Mean number of wells (x) ± SE, 2 groups of 6 animals. [d] Titre as obtained by 2^x. [e] Titre range as obtained from SE of x.

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Persistance of maternally derived ^{3}H-estradiol in fetal and neonatal rats[1]

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Summary. Injection of ^{3}H-estradiol into pregnant rats resulted in fetal blood radioactivity 5 times higher than in maternal blood. Significant amounts of ^{3}H-estradiol were found in fetal blood 24 h later and in the offspring 5 days after birth.

Distribution of ^{3}H-estradiol in fetal and neonatal rat tissues has been studied extensively[2–5], but no report on the long term retention of this steroid in the blood appears in the literature. Fetal and neonatal rat plasma contains an estradiol binding plasma protein (EBP) which has a capacity for binding large quantities of estradiol[6]. Its functional significance is not well understood. High concentrations are found in fetal rats, with a rising titer as they approach term. EBP displays a half-life in postnatal blood of about 4 days and disappears by the 29th day. EBP is found in low concentrations in pregnant rats, but is absent in nonpregnant adults[7]. Removal of ^{3}H-estradiol from the blood of nonpregnant adults is fairly rapid[8], but because of the presence of EBP one might suspect the retention of this steroid in the blood of fetal and neonatal rats could be longer.

Döhler and Wuttke found serum estradiol was greater in 1- and 2-day-old neonates than in any other age group, including adults[9]. Estradiol values declined slowly for several days, and then rose to moderately high levels by day 10. The authors were unable to explain this pattern. The declining estradiol levels parallel a known drop in circulating EBP and may be casually related to it. The estrogen in the offspring could result from placental transfer of maternal estrogen prior to parturition. The present study reports the long term retention of ^{3}H-estradiol in the blood of fetal and neonatal rats following injection of this steroid into the mother.

Materials and methods. Sprague-Dawley rats, 20 days pregnant, received i.v. 0.5 μCi ^{3}H-estradiol/g of b.wt. The estradiol (91.3 Ci/mM, New England Nuclear) was checked for purity by TLC prior to use. Injected animals were decapitated and their fetuses removed and kept cold until blood samples could be taken. Adult and fetal blood

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was solubilized with Soluene 350 and bleached with hydrogen peroxide. Specimens were counted in a Packard liquid spectrometer; counting efficiency was determined by the channel ratio method. Radioactivity was then expressed as DPM/µl of blood.

Other pregnant animals received s. c. 0.5 µCi/g b. wt of ^{3}H-estradiol on the morning of the 22nd day of gestation; animals that delivered within 5 h after the injection were used in the study. Pups were decapitated at various intervals from the time of injection and blood samples taken. Counting was done as before and expressed as DPM/µl of blood.

Results and discussion. 15 min after injection of ^{3}H-estradiol, radioactivity in fetal blood was 5 times higher than in maternal blood (figure 1). Fetal blood levels remained high over the initial 2 h, decreasing slowly. Maternal levels, however, lower to begin with, also dropped slowly. By 24 h, fetal blood radioactivity had dropped to about 10% of initial values but was still significantly higher ($p > 0.01$) than adult levels.

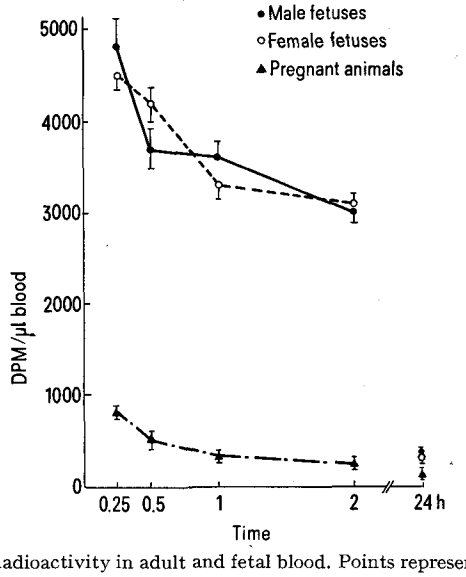

Fig. 1. Radioactivity in adult and fetal blood. Points represent mean ± SEM of 5 determinations for adult valves and 10 or more for fetal valves.

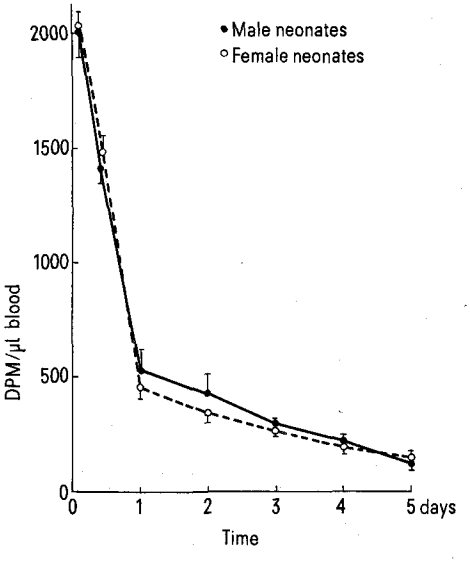

Fig. 2. Radioactivity in neonatal blood. Points represent mean ± SEM of 6 or more determinations.

Although the large dose of ^{3}H-estradiol was injected directly into the circulation of the mother, only small quantities of radioactivity remained after 15 min. It is obvious that shortly after the injection much of the ^{3}H-estradiol left the blood for various body compartments. This probably includes the liver, which can rapidly bind and metabolize steroids. The estrogen that remains is apparently bound to EBP, because significant amounts of radioactivity are found after 24 h. Larger amounts of radioactivity in fetal blood may have been the result of the greater concentration of EBP. In addition, the liver degrading system is not fully developed in fetal animals; maximum rates of steroid metabolism do not occur until 2–3 weeks after parturition[10].

Blood radioactivity values for neonatal animals are illustrated in figure 2. Although the mothers were injected with ^{3}H-estradiol several h before delivery, high levels were retained in neonates for the first 24 h. After the first day, blood levels gradually decreased and substantial amounts were still recorded in 5-day-old animals. No sex differences were observed.

Radioactivity extracted from plasma of fetal and neonatal rats was analyzed for ^{3}H-estradiol content by a reverse isotope dilution procedure[11]. Fetal plasma 24 h after maternal injections contained 22–31% unaltered ^{3}H-estradiol. Plasma from 3-h-old newborns had 23–36% and from 5-day-old rats had 18–36% of radioactivity as unaltered steroid. Thus, a substantial amount of the radioactivity was in another form, but significant amounts of ^{3}H-estradiol remained.

The blood values of radioactivity found in these neonates reflect the large dose of ^{3}H-estradiol injected. To determine whether these values were within the normal physiological range, the amout of estradiol was calculated from the radioactivity by assuming that only 25% was unaltered estradiol. 1 ml of blood from 1- and 5-day-old rats was estimated to contain 170 and 50 pg of estradiol, respectively. These values are in excellent agreement with the 300 and 70 pg of estradiol in 1- and 5-day animals as determined by radioimmunoassay[9].

Since some of the radioactivity extract from fetal and neonatal blood was identified as estradiol, and in view of the adult ovary's role of secreting this steroid during pregnancy[12], we conclude that maternal estradiol is circulating. The extended retention of estradiol in these animals is probably the result of the EBP that is present. Vannier and Raynaud proposed that EBP protects against the high estrogen levels of pregnancy[2]. Thus, estradiol is retained in the vascular bed and tissue damage is prevented. However, recent evidence suggests that estrogen may have important roles in the development of several tissues[13,14]. High estradiol levels may be required for various developmental processes. This study has demonstrated the presence of maternally derived ^{3}H-estradiol in a 5-day-old animal. Maternal estrogen may be maintained in neonatal animals until they are capable of producing their own steroid. This concept is supported by the fact that neonatal ovarian estrogen secretion begins on day 4[15] and there is an increase in serum estrogen levels between the 5th and 10th day of life[9,16].

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