Effects of passive immunization against oestradiol on mouse ovum transport

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Summary. The ability of anti-oestradiol immunoglobulin to withhold oestradiol-17 β from its target tissue was examined. The total oestradiol-17 β binding capacity present in in-vitro incubations or injected into mice intravenously was related to the amount of [3 H]oestradiol present in the media or intravenously injected into the animals respectively. When the ratio of binding capacity to [3 H]oestradiol was above 74:1, [3 H]oestradiol was successfully withheld from uterine tissue *in vitro* and *in vivo*.

Injecting anti-oestradiol immunoglobulin into mice before administration of a tubelocking dose of oestradiol-17 β ensured normal passage of ova through the oviduct. Daily administration of anti-oestradiol immunoglobulin to PMSG-hCG stimulated mice (starting 72 h before hCG injection) induced retention of ova for at least 2 days beyond the time when all ova had left the oviducts of control animals. The binding capacity: oestradiol-17 β concentration ratios of sera from these animals were > 250:1 throughout the experimental period. Non-specific immunoglobulin had no such effects, indicating the specificity of the anti-oestradiol immunoglobulin response.

Introduction

Early investigations revealed that oestrogenic substances are capable of preventing ovum transport through the oviducts of mice and rabbits (Parkes & Bellerby, 1926; Burdick & Pincus, 1935). Further examination of this effect showed that oestrogen could both accelerate and block the passage of ova through the mouse oviduct, depending upon the dose and the time of administration relative to the time of ovulation (Greenwald, 1961; Humphrey, 1976).

The effects of ovariectomy on tubal transport in mice have been inconsistent. Whitney & Burdick (1939) reported a prolonged period of ovum transport in mice ovariectomized on Day 1 of pregnancy, while Kendle & Lee (1980) found a normal transport rate under similar conditions. Ovariectomy on Day 2 of pregnancy caused oviducal retention of the majority of ova until Day 4 (Roblero & Garavagno, 1979): daily administration of oestradiol-17 β during this period resulted in the passage of most ova through the oviduct but there was also a substantial loss of ova through the uterus; progesterone injections yielded a more normal transport of eggs. Since there is an increase in progesterone production during the last part of oviducal transport in the mouse (McCormack & Greenwald, 1974; Barkley, Geschwind & Bradford, 1979), it appears that progesterone may be the factor that overrides the impediment to continued transport at this time. Although Kendle & Lee (1980) did not observe any effect of ovariectomy on Day 1 of pregnancy, they did inhibit ovum

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transport by administering antiprogesterational steroids on Days 1, 2 or 3. These results support the suggestion that post-ovulatory progesterone secretion may be important for normal ovum transport.

The present study attempts to clarify the role of oestrogen in ovum transport of the mouse.

Materials and Methods

Anti-oestradiol immunoglobulin. Anti-oestradiol-17 β serum from a ewe immunized against oestradiol-17 β -6-O-carboxymethyl(oxime)-BSA (Steraloids, Inc., New Hampshire) was partly purified by precipitation of non-immunoglobulin proteins with 0.4% 6,9-diamino-2-ethoxyacridine lactate (Sigma Chemical Co., St Louis, Missouri) according to the methods of Saifer & Lipkin (1959), followed by precipitation of immunoglobulins with ammonium sulphate. The final precipitate was redissolved in water and dialysed against water at 5–8°C for 48 h, freeze-dried and stored at –20°C.

Cross-reactivities of the anti-oestradiol immunoglobulin preparation were measured according to the method of Abraham (1974). Oestrone cross-reacted <7%; reactivities of other steroids of biological importance (progesterone, 20 α -dihydroprogesterone, oestriol, oestradiol-17 α , hydrocortisone, testosterone) were <1%. Diethylstilboestrol (Sigma) did not displace [3 H]oestradiol from the antibody even at molar concentrations 10000-fold that of the labelled ligand.

Partly purified immunoglobulins were prepared as above from serum of an ovariectomized sheep and used as the non-specific control protein.

Radiolabelled-oestradiol uptake studies. Uterine tissue for estimation of the effect of anti-oestradiol immunoglobulin on [3 H]oestradiol uptake was obtained from mature (60 days) female CD-1 mice (bred and housed in our animal facility) exhibiting dioestrous (leucocytic) vaginal smears. Tissue incubations were carried out at 37°C with shaking in 3 ml Krebs–Ringer–Henseleit buffer (KRH, as described by Jensen *et al.*, 1968) containing 50 μ g ovariectomized sheep serum immunoglobulin for the designated times. After cutting longitudinally to expose the luminal surface, one horn of each pair was placed in a flask containing 3×10^{-9} M-[3 H]oestradiol and the other in a flask containing 3×10^{-9} M-[3 H]oestradiol plus 3×10^{-5} M-diethylstilboestrol; each flask also contained a piece (50–100 mg) of diaphragm from the same animal. After incubation the tissue samples were rinsed four times in cold (4°C) KRH and gently blotted. Samples were weighed and dissolved in 1 ml tissue solubilizer (Protosol, New England Nuclear) at 55°C overnight. After cooling, the solubilized samples were decolorized by adding 100 μ l 30% H $_2$ O $_2$ and incubating for another 30 min at 55°C. After cooling again, 100 μ l glacial acetic acid and then 10 ml liquid scintillation fluid (Scintiverse, Fisher Scientific, Massachusetts) were added. Radioactivity was assessed on a scintillation counter and quench corrected by the external standard ratios method. Specific [3 H]oestradiol uptake was determined from the difference between uptake of tissues incubated in the absence and in the presence of diethylstilboestrol.

A preliminary time effect study had shown that maximal specific [3 H]oestradiol uptake was reached after incubation for 60–120 min. To study the effect of anti-oestradiol immunoglobulin on specific in-vitro uptake, tissues were incubated for 120 min as above with the addition of 0.05, 0.1, 0.2, 0.4, 0.8 or 2.0 mg of the specific immunoglobulin. Additional groups of tissues were incubated for 120 min with 0.40 mg ovariectomized sheep serum immunoglobulin to check for a non-specific effect, or with 0.10 mg anti-oestradiol immunoglobulin for 240 min to determine whether its effect was time dependent.

Oestradiol uptake *in vivo* was examined in mice 15 or 60 min after an i.v. (tail vein) injection of 18 ng [3 H]oestradiol with or without prior administration of a 1000 M excess of diethylstilboestrol. In a preliminary experiment, the estimate of specific uptake reached a maximum within 15 min after the [3 H]oestradiol injection. Diethylstilboestrol was administered in 50 μ l ethanol:propylene glycol:water solution (8:40:52, by vol.); [3 H]oestradiol was delivered in 50 μ l of a 0.1%

ovariectomized sheep serum immunoglobulin, 0.9% (w/v) NaCl solution. Vehicle was injected into animals that did not receive a dose of diethylstilboestrol. At 15 min before the [³H]oestradiol injection, the mice received an i.v. injection of 1, 2.5 or 5 mg anti-oestradiol immunoglobulin in 100–200 µl 0.9% NaCl solution. After death by decapitation, uterine and diaphragm samples were excised, rinsed, and solubilized as in the in-vitro uptake studies.

Radioimmunoassays. Oestradiol-17β and progesterone assays were performed according to procedures outlined by Overstrom, Bigsby & Black (1980) with the following modifications. When serum from passively immunized animals was to be assayed for oestradiol-17β it was subjected to treatment with guanidine hydrochloride (Sigma) at a rate of 100 mg/100 µl serum to improve the extractability of the steroid. When progesterone and oestradiol-17β were both assayed in the same sample, the ether extracts were submitted to Sephadex LH20 chromatography as described by Orczyk, Caldwell & Behrman (1974). To account for procedural losses small amounts (2–5 pg) of [³H]oestradiol and [³H]progesterone were added to each of these samples before guanidine treatment and ether extraction.

In addition to the assay validations for rabbit serum described by Overstrom *et al.* (1980), the performance of both assays was evaluated for mouse serum. Assay of increasing volumes of a mouse serum pool yielded linear relationships for each steroid described by the equations: $y = 16.8x - 2.56$ (y = pg oestradiol-17β, x = ml/sample), with a correlation coefficient of $r = 0.986$ for 6 samples, and $y = 16.0x + 1.54$ (y = ng progesterone, x = ml/sample), $r = 0.985$ for 11 samples. Addition of 10 or 20 pg of oestradiol-17β to triplicate samples of the same pool resulted in assayed amounts of 12 ± 0.5 pg and 21 ± 2.0 pg (mean $\pm$ s.d.), respectively, after correction for endogenous oestradiol-17β.

The oestradiol-17β assays were sensitive to 2–6 pg per extraction and the progesterone assays had sensitivities of 8–26 pg per extraction.

Binding capacities. Serum from passively immunized animals was analysed for total oestradiol-17β binding capacity. Each serum sample was diluted serially with the gelatinized phosphate-buffered saline used in the radioimmunoassays. A constant volume (0.1 ml) of each dilution was reacted with 20000 c.p.m. [³H]oestradiol. After 4–24 h at 5–8°C, the bound and free [³H]oestradiol were separated by the dextran-coated charcoal technique. The 3 dilutions which yielded proportional amounts of specifically bound [³H]oestradiol (corrected for the amount bound to corresponding dilutions of a non-immune control sample) were used to calculate the binding capacity. Proportionality between dilutions was usually achieved when the bound fraction represented 10–40% of the total [³H]oestradiol present. Binding capacity for the sample was calculated for each dilution according to the following equation:

$$B = \frac{I - N \text{ (c.p.m.)}}{0.1 \text{ ml}} \times \frac{1}{K} \times \text{sp. act. (c.p.m./ng)}$$

where B = total binding capacity in ng/ml, I = total c.p.m. bound for the immune sample, N = c.p.m. bound of the non-immune sample, and K = the dilution as a fraction. The average of the 3 dilutions was used.

Transport studies. Mature (60 day, CD-1) mice were selected at random and housed 2 per cage during the experimental period. They were kept in an air-conditioned environment with 14 h light/24 h (05:00–19:00 h). Food (Laboratory Rodent Chow, Purina) and water were available *ad libitum*. Daily vaginal lavages were performed and the stage of the oestrous cycle was determined from microscopic examination of the smears produced. Smears were examined between 15:00 and 17:00 h.

The ability of anti-oestradiol immunoglobulin to block the effects of exogenous oestradiol-17β on ovum transport was examined by administering oestradiol-17β alone or with the immunoglobulin on the morning following mating of gonadotrophin-primed mice. Animals selected at random

were injected i.p. with 4 i.u. PMSG (Sigma) in the afternoon; 48 h later 4 i.u. hCG (Sigma) was administered i.p. Each mouse was then housed with a male of proven fertility. The next morning (Day 1) animals with a vaginal plug were injected with 1.8 mg anti-oestradiol immunoglobulin (in 0.18 ml 0.9% NaCl solution, i.p.) followed 2 h later by 400 ng oestradiol-17 β in peanut oil (s.c.) or with oestradiol-17 β alone; control mice received a s.c. injection of peanut oil. Groups of 5 mice were killed at Days 3, 4 and 5 to examine the numbers and locations of the eggs.

To study the effects of anti-oestradiol immunoglobulin on egg transport, antibody treatment was begun before the priming dose of PMSG. Since the withdrawal of circulating oestradiol-17 β was likely to prevent the normal mating response in these animals, they were not housed with males after the hCG injection. To ensure synchrony of ovulation among animals, the PMSG was given to each animal 24 h after the appearance of cornified epithelial cells in smears of their vaginal lavages (indicating the onset of, at the least, the second consecutive pro-oestrus). Control animals received only the PMSG and hCG treatments between 16:00 and 18:00 h. Mice received daily doses of 1 mg anti-oestradiol immunoglobulin i.p. in 0.1 ml 0.9% NaCl starting the day before PMSG. An additional group of control animals received daily i.p. injections of 1 mg ovariectomized sheep serum immunoglobulin, again starting the day before PMSG. Groups of animals were killed by decapitation at 48, 72 and 96 h after hCG. Blood was collected from each animal at the end of the experimental period and the serum was stored at -20°C until analysis for oestradiol-17 β and progesterone concentration and oestradiol-17 β binding capacity.

The location of ova within the reproductive tracts was determined by flushing the oviducts, uterine horns, and vagina with a saline solution. Each flush was examined under a dissecting microscope for the presence of ova.

Statistical analysis. Differences between means were analysed by a least squares analysis of variance method. The analysis was performed at the University of Massachusetts Research Computing Centre utilizing a program entitled G4 UMASS, UNEQFREQ—Least Squares Analysis of Variance for Unequal Subclasses.

Results

The efficacy of the anti-oestradiol immunoglobulin was shown by its ability to block the effect of exogenous oestradiol-17 β on ovum transport. After a single injection of 400 ng oestradiol-17 β on the first day of pregnancy, 60 and 47% of recovered ova (totals 33 and 23) were found in the oviducts on Days 4 and 5 respectively. Oviducts of Day-3 and Day-4 control animals contained 40 and 3% of the recovered ova respectively (totals 59 and 35), but no ova were flushed from the oviducts or uterine horns on Day-5. Animals that were treated with both anti-oestradiol immunoglobulin and oestradiol-17 β had only 5% of their 47 ova in the oviducts on Day 4; on Day 5 there were no oviducal ova, but ova were flushed from the uterus.

Anti-oestradiol immunoglobulin reduced the amount of specific [^3H]oestradiol uptake *in vitro* in a dose-related manner (Table 1); the uptake was completely blocked with 0.80 mg. The ratio of total binding capacity to [^3H]oestradiol content of the 0.8 mg flask was approximately 74:1; a ratio of 18:1 (0.2 mg) reduced the specific uptake by about 98%. The inhibition of uptake was not influenced by the duration of the incubation. When 0.1 mg anti-oestradiol immunoglobulin was added to the flasks and incubated for 4 h the specific uptake, 1.2 ± 0.23 fmol/mg, was nearly identical to that observed in the 2-h incubation (Table 1).

At 15 min after injecting 18 ng [^3H]oestradiol i.v. both total and specific uterine uptake reached a zenith (data not shown). Anti-oestradiol immunoglobulin induced a dose-related decrease in the total uptake. When animals were pretreated with 5 mg anti-oestradiol immunoglobulin there was no longer a difference between uterine uptake of the diethylstilboestrol-treated (non-specific) and vehicle-treated (total) groups (Table 2). The ratio of total binding capacity to total [^3H]oestradiol injected in the 5 mg anti-oestradiol immunoglobulin group was approximately 108:1.

Table 1. Uptake of [³H]oestradiol after 2 h by mouse uterus *in vitro*

Anti-oestradiol immunoglobulin (mg)	Ratio of binding capacity: oestradiol†	Specific uptake (fmol/mg)
0*	—	27.3 ± 3.20 (6)
0.05	4.6	2.6 ± 0.02 (3)
0.10	9.3	1.2 ± 0.08 (3)
0.20	18.5	0.55 ± 0.16 (3)
0.40	37	0.33 ± 0.13 (3)
0.80	74	0.07 ± 0.09 (3)
2.00	185	— 0.08 ± 0.07 (3)

Values are mean ± s.e.m., no. of mice per group in parentheses.

* The incubation flasks contained 0.4 mg ovariectomized sheep immunoglobulin.

† Ratio of total oestradiol binding capacity to total [³H]oestradiol present in incubation flasks.

Table 2. Uptake of [³H]oestradiol by mouse uterus *in vivo* after treatment with anti-oestradiol immunoglobulin

Amount of Ig (mg)	Uterine uptake (fmol/mg)		
	Total	Non-specific	Specific
0	11.9 ± 1.28 (5)	1.45 ± 0.13 (4)	10.5
1	2.23 ± 0.22 (3)	0.99 ± 0.08 (3)	1.24
2.5	1.86 (1)	—†	—†
5.0	0.99 ± 0.33 (2)	0.97 ± 0.07 (3)	0

Values are mean ± s.e.m., the no. of mice per treatment in parentheses.

† Not examined.

When *in-vivo* uptake was examined at 60 min in anti-oestradiol immunoglobulin-treated mice, the non-specific uptake was actually higher than the total uptake (data not shown). Such a discrepancy is apparently due to the increased vascular permeability following oestrogenic stimulation by the large dose of diethylstilboestrol. Since it has been shown that there is a minimum lag of 30 min after intravascular oestrogen administration before the onset of a vascular response (Killam, Rosenfeld, Battaglia, Makowski & Meschia, 1973; Arvidson, 1977; Clewell, Carson & Meschia, 1977), it is unlikely that vascular perturbations affected the results of the 15-min uptake studies *in vivo*.

During the entire experimental period of the ovum transport studies, serum binding capacity to oestradiol-17β concentration ratios were well above the 74:1 ratio determined to be adequate for complete inhibition of specific oestradiol-17β uptake *in vitro* (Table 3).

The pattern of progesterone concentrations of the pooled serum samples from the anti-oestradiol immunoglobulin-treated animals did not appear to differ from that exhibited by control animals (Table 4). However, because assay of the steroids required pooling of serum samples any differences among groups or individuals could not be assessed statistically.

The effect of passive immunization against oestradiol-17β on transport of ova through the mouse oviduct is presented in Table 5. By 72 h after hCG nearly all ova had entered the uteri of control animals. A decrease in the total number of ova found within the tract also occurred at 72 h and again at 96 h. When similar animals were treated with anti-oestradiol immunoglobulin a

Table 3. Binding capacities, oestradiol-17 β concentrations, and binding capacity:oestradiol-17 β ratios

Time (h)*	Binding capacity† (ng/ml)	Oestradiol conc.‡ (ng/ml)	Ratio of binding capacity: oestradiol
-68	68.4 $\pm$ 8.0	0.20	342
-50	55.2 $\pm$ 8.8	0.21	263
-46	129 $\pm$ 30.6	0.18	717
+24	119 $\pm$ 3.4	0.26	458
+48	303 $\pm$ 25	1.06	285
+72	360 $\pm$ 20	0.64	567
+96	239 $\pm$ 29	0.87	275

* The time of hCG injection was 0 h. Except for values at -68 and -46 h the data were collected from serum obtained before the next scheduled anti-oestradiol immunoglobulin injection.

† Values are mean $\pm$ s.e.m. for 3 animals per group at -68 h to +24 h and 5-6 animals per group from +48 to +96 h.

‡ The mean of duplicate determinations.

Table 4. Oestradiol (E₂) and progesterone (P) serum concentrations from treated and control mice

Time after hCG (hours)	Control		Non-specific immunoglobulin		Anti-oestradiol immunoglobulin	
	E ₂ (pg/ml)	P (ng/ml)	E ₂ (pg/ml)	P (ng/ml)	E ₂ (pg/ml)	P (ng/ml)
48	<5	31.5	<5	9.8	1060	12.3
72	<5	61.1	<5	26.1	640	64.3
96	8.3	20.3	<5	81.3	870	31.3

Serum was pooled from each treatment group and duplicate samples of each pool were assayed; the mean of these two samples is presented.

Table 5. Numbers and position of ova at 48, 72 and 96 h after hCG in mice treated with anti-oestradiol immunoglobulin (Ig)

Treatment	No. of mice	Time after hCG (h)	Numbers of ova*	
			Oviduct	Uterus
Control	15	48	15.6 $\pm$ 3.71 (81)	3.7 $\pm$ 1.66 (19)
Non-specific Ig	5	48	8.6 $\pm$ 3.03 (59)	6.0 $\pm$ 2.81 (41)
Anti-oestradiol Ig	6	48	25.3 $\pm$ 2.22 (97)	0.8 $\pm$ 0.83 (3)
Control	10	72	0.6 $\pm$ 0.50 (9) ^a	5.8 $\pm$ 1.96 (89)
Non-specific Ig	5	72	0	5.6 $\pm$ 3.04 (100)
Anti-oestradiol Ig	6	72	12.5 $\pm$ 6.97 (46) ^b	14.5 $\pm$ 5.60 (54)
Control	10	96	0.8 $\pm$ 0.70 (33) ^a	1.6 $\pm$ 0.64 (67)
Non-specific Ig	5	96	0	4.2 $\pm$ 1.74 (100)
Anti-oestradiol Ig	5	96	15.4 $\pm$ 9.10 (90) ^b	1.4 $\pm$ 0.75 (8)

Values are mean $\pm$ s.e.m. ova per mouse (%).

Means within a column at each time with different superscripts are significantly different ($P < 0.05$).

* Only 2 groups contained vaginal eggs.

considerable number of eggs remained in the oviducts at 72 and 96 h. Also, at 72 h more than twice as many eggs were found in the uteri of anti-oestradiol immunoglobulin-treated animals than non-treated or non-specific immunoglobulin treated controls. No eggs were found in the oviducts of non-specific immunoglobulin-treated control mice at 72 or 96 h, indicating the specificity of the anti-oestradiol effect.

Discussion

Immunoglobulins against oestradiol-17 β were capable of completely inhibiting specific [^3H]oestradiol uptake *in vitro* when the total binding capacity of the antibody present was 74-fold or greater than the amount of [^3H]oestradiol in the medium (Table 1). Likewise, administration of anti-oestradiol immunoglobulin with a binding capacity of 108-fold the amount of [^2H]oestradiol administered *in vivo* totally inhibited the uptake of the steroid by the uterus (Table 2). The injection of anti-oestradiol immunoglobulin and [^3H]oestradiol at a ratio of approximately 22 : 1 resulted in an 88% decrease in specific uptake *in vivo*; this compares to a 98% decrease produced by a ratio of about 18 : 1 *in vitro* (Table 1, Table 2). Thus, the *in-vivo* and *in-vitro* estimates of the anti-oestradiol immunoglobulin effect were comparable. Ratios for binding capacity:oestradiol-17 β content yielding total inhibition of specific uptake may therefore be used as a guide in evaluating the capacity of circulating antibody concentrations to inhibit target tissue uptake of circulating oestradiol-17 β , thereby neutralizing endogenous sources of that steroid.

Oestradiol-17 β binding capacities in the serum of passively immunized mice were greater than 250-fold the oestradiol-17 β concentrations throughout the experimental period (Table 3), indicating that the amount of anti-oestradiol immunoglobulin in those mice was sufficient to block target tissue uptake of endogenous oestradiol-17 β .

In naturally ovulating, mated mice entry of ova into the uterus begins 66–69 h after coitus and continues until 81 h *post coitum*, the majority of ova remaining in the oviducts up to 78 h (Humphrey, 1968). In the present study, within 72 h after administration of hCG to PMSG-primed mice 91–100% of the ova had left the oviducts of control animals (Table 5). Once in the uterus the eggs were rapidly expelled through the tract, as indicated by the decrease in the total number of ova recovered per mouse at 72 and 96 h after hCG. Ovum transport in the gonadotrophin-primed, cyclic mouse is therefore more rapid than in mated animals; the reason for this difference is unknown. Hodgson & Pauerstein (1976) observed that ova entered the uteri of hCG-stimulated rabbits earlier than those of unstimulated, mated animals. The number of ova observed in all treatment groups at 48 h indicates that the gonadotrophin regimen used induced superovulation (Table 5). It may be that transport is accelerated in animals that have been overstimulated with PMSG. The degree of transport acceleration observed in the gonadotrophin-primed, unmated mice of the present study was greater than that observed by Lungu (1980) in gonadotrophin-primed, mated mice, indicating that, in addition to the effect of superovulation, re-entry into the ovarian cycle affects the transport of eggs in the mouse. Such differences among transport rates in normal mated mice, superovulated mated mice, and superovulated unmated mice confound the interpretation of the present data, making it difficult to extrapolate conclusions to the mechanisms of transport in normal pregnancy.

Humphrey & Martin (1968) and Shingh & Kamboj (1981) interpreted the tube-locking effect of synthetic antioestrogens as an indication of their inherent oestrogenicity. Oestrogens, however, simultaneously cause tube-locking and uterine expulsion of ova (Greenwald, 1961; Humphrey & Martin, 1968; Humphrey, 1976). The lack of expulsion of ova from uteri of mice treated with synthetic antioestrogens (Humphrey & Martin, 1968; Thomson, 1968; Humphrey, 1976) or with anti-oestradiol immunoglobulin (Table 5) suggests that the effect of the synthetic antioestrogens was indeed antioestrogenic.

It cannot be ascertained from the present study whether pre-ovulatory and/or post-ovulatory

oestrogen is important in normal ovum transport. Since effects similar to those reported here were observed when synthetic antioestrogens were administered after ovulation (Humphrey & Martin, 1968; Thomson, 1968; Humphrey, 1976; Shingh & Kamboj, 1981), it seems likely that it is the post-ovulatory oestrogens which are important. Lungu (1980) found a decreased ovum transport rate in mice treated with a single i.p. injection of anti-oestradiol serum at the time of hCG injection, but there was only a marginal delay in transport in that study, perhaps because of the manner in which the antiserum was administered. Further studies using mated animals treated with anti-oestradiol immunoglobulin on Days 1, 2 and 3 will permit clarification of the involvement of post-ovulatory oestrogen is required for normal transport.

Roblero & Garavagno (1970) concluded that oestradiol-17 β is necessary for ovum movement through the mouse oviduct and that progesterone is necessary to counteract the effect of oestradiol-17 β by depressing uterine contractile activity (Porter, 1968; Csapo, 1970; Aref & Hafez, 1973; Spilman & Wilks, 1976). In the present study, progesterone levels of the anti-oestradiol immunoglobulin-treated mice were high at 72 h after hCG but fell by 96 h; the decrease in progesterone may be the cause of uterine expulsion of ova at 96 h in these animals. Indeed, there was a tendency for the non-specific immunoglobulin-treated controls, which exhibited the high progesterone levels at 96 h, to have more ova remaining in their uteri than did the other two treatment groups (Table 5). Hence, findings in the mouse reported here support Roblero & Garavagno's (1979) suggestion that oestradiol-17 β and progesterone are important in controlling oviducal transport and uterine quiescence, respectively.

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