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An Attempt to Demonstrate the Presence of a Local Utero-Ovarian Transport Mechanism in the Guinea Pig by Radioisotope Tracer Techniques

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AN ATTEMPT TO DEMONSTRATE THE PRESENCE OF A LOCAL UTERO-OVARIAN TRANSPORT MECHANISM IN THE GUINEA PIG BY RADIOISOTOPE TRACER TECHNIQUES

by

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Presentation of the Problem

Species with bipartite uteri such as the bovine and ovine, with bicornuate uteri such as the porcine, and with duplex uteri such as the guinea pig respond to hysterectomy by having prolonged life-spans of corpora lutea and by demonstrating estrual suppression (Ginther, 1967). Furthermore, unilateral hysterectomy in these species, immediately following estrus, results in extended life-spans of corpora lutea located on the ovary ipsilateral to the removed uterine horn, while the life-span of the corpora lutea located on the ovary contralateral to the removed uterine horn is unaffected (du Mesnil du Buisson, 1961; Ginther, Woody, Mahajan, Janakiraman and Casida, 1966b; Inskeep and Butcher, 1966; Fischer, 1965). Upon removal of one uterine horn in rats, luteal activity responds similarly to the above mentioned species but only after pseudopregnancy has been induced (Melampy, Anderson and Kragt, 1964).

Results of these studies imply an interplay between the uterus and ovary and more specifically interaction between a uterine horn and the corpora lutea of ovaries located adjacent to that uterine horn. Many investigators (Ginther et al., 1966b; Inskeep et al., 1966; Fischer, 1965; Bland and Donovan, 1965; Williams, Johnston, Lauterbach and Fagan, 1967) favor the theory that the uterine horns produce a luteolytic substance (uterine luteolytic factor, ULF) which is transferred to the adjacent ovary without passing through the general

circulation. With the absence of ULF, resulting from the removal of a uterine horn, corpora lutea life-spans are extended. However, no anatomical or physiological communication between ipsilateral uterine horn and ovaries by which ULF could reach the ovary has been demonstrated. Therefore, the present study was undertaken to test if such a local transport between uterine horns and ovaries on the same side in guinea pigs existed. Radioisotope tracer studies were performed in which tritium labeled acetate or estradiol-17β were quantitatively determined in various tissues of the body following intrauterine injection of the radioisotopic compound. Prior to intrauterine injection, ligations of various veins and arteries associated with the reproductive organs were made. Tissues were assayed two minutes to two hours after isotope injection on the groups of animals subjected to the various occlusions.

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LITERATURE REVIEW

Loeb reported in 1923 and again in 1927 that a suppression of estrus for 50 to 100 days followed hysterectomy in the guinea pig. Since the duration of the estrous cycle in the intact female guinea pig is approximately 16.5 days, Loeb concluded that hysterectomy extends the length of the estrous cycle. These observations in the guinea pig were later substantiated by many other investigators (Rowlands and Short, 1959; Rowlands, 1961; Butcher, Chu and Melampy, 1962b; Deanesly and Perry, 1965). Hysterectomy in bovine, ovine and porcine also results in estrus suppression (Willbank and Casida, 1956; Anderson, Butcher and Melampy, 1961). The estrous cycle is unaffected following hysterectomy in normally cycling monkeys, marsupials, ferrets, opossums, rats and rabbits (Burford and Diddle, 1936; Deanesly and Parkes, 1933; Hartman, 1925; Bradbury, 1937; Asdell and Hammond, 1933). Prolonged estrus suppression is observed in rats and rabbits following hysterectomy only if pseudopregnancy precedes uterine horn removal (Bradbury, 1937; Asdell *et al.*, 1933).

Suppression of estrus is accompanied by the absence of ovulation. Anovulation was demonstrated by nearly all of the investigators either by directly observing only the corpora lutea formed prior to uterine removal, or indirectly by observing the presence of a deeply mucified vaginal epithelium. Along with the suppression of estrus and the absence of ovulation following hysterectomy, corpora lutea formed prior to uterine removal are present through the extended diestrus phase. Furthermore, these tenacious corpora lutea achieve a size

equal to or greater than the size of a corpus luteum during pregnancy (Loeb, 1927; Rowlands, et al., 1959; Rowlands, 1961; Willbank, et al., 1956; Collins, Inskeep, Howland, Pope and Casida, 1966). Actually the increase in size suggests increased progesterone production but this was not known to be the case until Rowlands and Short (1959) quantitatively measured the progesterone concentration in the blood and in the corpora lutea. Since high titers of progesterone prevent ovulation (Nalbandov, 1964) and progesterone production is a function of the corpus luteum, high levels of progesterone concentration in the corpus luteum probably indicate an anovulatory condition.

With the acceptance of prolonged luteal activity and suppression of estrus following hysterectomy, investigators began studies with subtotal hysterectomies. Loeb (1927) observed a prolonged diestrus and corpora lutea maintenance when two-thirds to three-fourths of both uterine horns were removed in the guinea pig. The prolongation was longer than that in intact guinea pigs but much shorter than that exhibited following complete hysterectomy. Later Butcher et al., (1962b) observed a correlation between removal of various amounts of uterine tissue in guinea pigs and luteal retention and diestrus extension. When both uterine horns were removed, estrus did not return for at least 63 days and all pre-formed corpora lutea persisted (estrous cycle of guinea pig is 16.5 days). If only the uterus posterior to the external bifurcation or if just the anterior one-fourth of both uterine horns and the cervix were left intact, estrus reoccurred but at 60 day cycles. This same effect was observed with removal of one uterine horn posterior to the external bifurcation and one-fourth

of the posterior part of the other horn or with only the anterior half of one horn left intact and the rest of the uterus removed. With one uterine horn removed more regular cycles of 20 to 25 days were observed. The corpora lutea maintained in hysterectomy and subtotal hysterectomy mimicked the corpora lutea of pregnancy in size and progesterone content. A striking feature in their work was that the guinea pigs exhibiting estrous cycles of less than 32 days per cycle were those animals with large amounts of uterine endometrium remaining. Similar responses to varying degrees of subtotal hysterectomy were observed in the sow (Anderson et al., 1961), heifer (Anderson, Neal and Melampy, 1962), ewe (Moor and Rowson, 1966a) and pseudopregnant rat (Silbiger and Rothchild, 1963).

When guinea pigs, cows, ewes, sows and pseudopregnant rats were unilaterally hysterectomized, the corpora lutea formed on the ovary located adjacent to the removed uterine horn were maintained for a period exceeding normal corpus luteum life-span characteristic of that animal. The corpora lutea located in the ovary contralateral to the removed uterine horn exhibited normal life-spans characteristic of the animal (Ginther, 1966). Many other studies were performed that demonstrate local effects between uterine horns and corpora lutea in adjacent ovaries. Pregnancy in one uterine horn of the pig (Anderson, Rathmacher and Melampy, 1966; Ginther, 1967) and guinea pig (Oxenreider and Day, 1967) results in maintenance of corpora lutea on the ovary adjacent to the pregnant uterine horn. Intrauterine plastic coils inserted into one uterine horn in the guinea pig (Ginther, Mahajan and Casida, 1966a), ewe (Ginther, Pope and Casida, 1965;

Stormshak, Lehman and Haek, 1967) and heifer (Ginther, Woody, Janakiraman and Casida, 1966c) result in enhanced regression of corpora lutea on the operative side. Unilateral insertion of glass beads into one uterine lumen of the pig mimics the results obtained with unilateral insertion of a plastic coil (Ginther, 1966). When embryos are transferred to one uterine horn in sheep, corpora lutea are functional on the adjacent ovary throughout the entire gestation period (Moor and Rowson, 1966b). Administration of oxytocin in intact heifers reduces the corpora lutea life-spans but injection of oxytocin in unilaterally hysterectomized heifers is ineffective in reducing the corpora lutea on the affected side while the corpora lutea on the unaffected side exhibit precocious regression (Armstrong and Hansel, 1959; Anderson, Bowerman and Melampy, 1965; Ginther et al., 1966b). In all the cases mentioned above, with the exception of oxytocin injection, the corpora lutea on the ovary contralateral to the affected uterine horn exhibit life-spans characteristic of non-treated animals. Suppression of estrus and ovulation is associated with unilateral retention of corpora lutea (Ginther, 1966).

With the accumulation of evidence it became apparent that the uterus, or part of the uterus, is involved with corpora lutea maintenance. Local interaction between uterine horns and corpora lutea located on the ovaries adjacent to the uterine horns is also implied.

Since the uterus, and especially the uterine endometrium, changes both physiologically and anatomically during estrous cycles, studies were performed to evaluate the effect of hysterectomy at different stages during the estrous cycle on the corpora lutea life-span and

estrus suppression. Rowlands (1961) hysterectomized guinea pigs on the fifth, tenth and fifteenth day after ovulation. Hysterectomy on the fifth day resulted in enlarged corpora lutea containing high levels of progesterone (180 µg per corpus luteum). Also, ovulation and estrus were suppressed for at least two months following hysterectomy. Hysterectomy on the tenth day after ovulation enlarged the corpora lutea to a size comparable to those of pregnant guinea pigs, but smaller than those corpora lutea of the animals hysterectomized on day 5 after estrus. The regression time of corpora lutea in these hysterectomized guinea pigs mimicked regression time for the corpora lutea of the animals hysterectomized five days after ovulation. Estrus suppression was also similar in the two groups of hysterectomized animals. Hysterectomy on the fifteenth day after ovulation resulted in life-spans of corpora lutea and estrous cycles resembling those of intact animals. Rowlands concluded that removal of uterine tissue before the tenth day after ovulation in the guinea pig affects the corpora lutea life-spans and the estrous cycle. Anderson et al. (1962) observed extended corpora lutea life-spans and estrous cycle alterations in bovine if hysterectomy was performed between the eighth and eleventh day following ovulation while Collins et al. (1966) observed changes in the estrous cycle and extended life-spans of corpora lutea in ewes only if hysterectomy was performed prior to 5.5 days after ovulation. Uterine removal before day sixteen of estrus in pigs and day eleven in pseudopregnant rats (Anderson, Butcher and Melampy, 1963; Melampy et al., 1964) results in extended corpora lutea life-spans.

In other studies, uterine preparations were either injected or transplanted into intact or hysterectomized animals. Butcher, Chu and Melampy (1962b) autotransplanted cross-section slices, endometrial scrapings, and chopped or whole uterine horns five days after ovulation in the guinea pig. Of 18 animals receiving a trace of endometrium, four showed estrus at days 50, 51, 69 and 72 days after pre-operative estrus while the other 14 animals returned to estrus at least 75 days after pre-operative estrus. Thirteen more animals were then hysterectomized on the fifth day of ovulation. Uterine autotransplants, which contained large amounts of endometrium, were injected subcutaneously. Two animals never returned to estrus, three animals had cycle lengths of 53, 57 and 65 days, while the rest of the guinea pig's post-operative cycle lengths ranged from 16 to 46 days. Bovine endometrial extracts in hysterectomized pseudopregnant rats were ineffective in reducing pseudopregnancy (Malven and Hansel, 1965). Anderson et al., (1963) observed no suppression of estrus in pigs following uterine autotransplants. Postmortum examination in the uterine autotransplanted pigs showed a lack of uterine endometrial presence. Acetone dried bovine endometrial powder (late luteal or early estrus) was injected intraperitoneally into pseudopregnant rabbits (Williams et al., 1967). Sixty-four percent of the rabbits exhibited regression of corpora lutea. When the corpora lutea-rich ovary was studied in vitro with the uterine bovine extract, acetate incorporation into progesterone was decreased when compared to untreated preparations. Similar in vitro results were obtained when guinea pig uterine extracts were mixed with guinea pig ovaries containing corpora lutea (Cooper

and Hess, 1965). The data demonstrates a dramatic inhibition of progesterone biosynthesis. On the other hand, when guinea pig uterine extracts were mixed in vitro with rat ovaries heavily laden with corpora lutea, transformation of pregnenolone to progesterone was unaltered.

With the exception of the experiments involving rat ovaries subjected to guinea pig uterine extracts, all the studies in which exogenous uterine endometrium was administered suggest a relationship between the uterus and ovary and a local interaction between adjacent uterine horns and ovaries. Furthermore, many investigators believe a substance is produced by the uterine endometrium that acts as a luteolytic agent on the corpora lutea. The mechanism of transport of this theorized substance has not been established. Anatomically no blood or lymph flows directly between uterine horns and ovaries (Greene, 1959; Roddenberry and Allen, 1967). Roddenberry and Allen demonstrated a lack of lymphaticovenous anastomosis in the abdominal region. Since the uterine horns and fallopian tubes are continuous and the tubes are in close proximity to the ovary, diffusion of the uterine luteolytic substance has been hypothesized. When the oviduct was ligated in intact guinea pigs and gilts, corpora lutea life-spans were unaffected. (Rowlands 1961; Butcher et al., 1962a; Anderson et al., 1961). If the cervix, uterine body, uterine horns and posterior halves of the oviducts are removed, the bovine does not return to estrus (Anderson et al., 1963). When the oviducts were removed from normally cycling ewes, the life-spans of the formed corpora lutea were the same as the life-spans of corpora lutea in intact ewes

(Kiracofe and Spies, 1963). In the unilateral pregnant guinea pig, removal of the fallopian tube on the side of pregnancy has no effect on the life-span of the corpora lutea on the ovary ipsilateral to the pregnant uterine horn. These corpora lutea are maintained throughout pregnancy (Deanesly, 1967).

With evidence negating transport of a substance from uterine horn to ovary by any known anatomical connections, the present study was undertaken to assess indirectly the existence of a possible local communication between ipsilateral uterine horn and ovary in the guinea pig by a radioisotope tracer technique.

METHODS AND MATERIALS

Experimental Animals

Sexually mature, nulliparous, female guinea pigs ranging in weight from 210 to 900 grams were used in this study. The guinea pigs were obtained from a commercial supplier (Kuiper Rabbit Ranch, Gary, Indiana).

Prior to their use, the guinea pigs were housed in a physiology teaching laboratory (Western Michigan University) and subjected to diurnal cycles of fourteen hours of light and ten hours of darkness each day. Room temperature was held nearly constant at 23°C and the guinea pigs were given water and Guinea Pig Chow (Purina) ad libitum supplemented with either lettuce or cabbage, weekly. At the time of use all guinea pigs were in the diestrus phase of the estrous cycle as determined by observing closed vaginas and uterine horns devoid of extensive vascularization.

Chemicals

Two radioactive chemicals were used in this research. They were tritiated acetate (labled on C-1) and estradiol-17β (tagged at the 7-alpha position). Both of the radioisotopic compounds were products of New England Nuclear Corporation. Each had a specific activity of 1 mc/mg and a concentration of 100 μc/ml of saline solution (0.85 per cent NaCl). Prior to use the radioactive solutions were further diluted in saline to a concentration of 15 μc/ml. A dye, dianil blue

(Hartman-Leyden Company), was used in part of the study along with the radioisotopic solutions; it was used as a 1 per cent solution in deionized water.

Surgical Procedures

Guinea pigs were weighed and anesthetized with intraperitoneal injections of sodium pentabarbitol (Diabutal, Diamond Laboratories Inc.). Dosage was 25 mg/kg body weight. A mid-ventral incision was made and the uterus and ovaries were exposed, at which time one of the following surgical procedures was performed (see Table 1).

1. The anterior uterine vein was occluded immediately posterior to its junction with the ovarian vein and the posterior uterine vein was occluded immediately anterior to the branches to the cervix. Both ligations were made on the same side of the animal with nylon thread.

2. Occlusions were performed as mentioned above plus the uterine arteries associated with the occluded veins were ligated.

3. The ovarian branches of the ovarian artery, just prior to entering the ovary, were ligated on one side of the animal.

4. The ovarian branches of the ovarian artery, just prior to entering the ovary, were ligated on both sides of the animal.

5. No ligations of blood vessels were performed.

Upon completion of the surgical manipulation, 0.3 ml (4.5 μc) of tritiated acetate or estradiol-17β was injected via the cervix into the uterine lumen on the side subjected to the surgical manipulation. Compacts containing heated physiological saline solution were then placed on the open wound in an attempt to maintain the body tempera-

Table 1: Various occlusions of the utero-ovarian vasculature.

Surgical Experiment	Time	Acetate	Estradiol	Dianil Blue
1. Unilateral occlusion of anterior and posterior uterine vein.	30 Min.	x	-	-
2. Unilateral occlusion of anterior and posterior uterine vein and associated artery.	30 Min.	x	-	-
3. Unilateral occlusion of ovarian branches of ovarian vein.	30 Min.	x	x	-
4. Bilateral occlusion of ovarian branches of ovarian arteries.	2 Min.	-	x	Every animal
"	6 Min.	-	x	Every other animal
"	10 Min.	-	x	Every other animal
"	15 Min.	x	-	-
"	30 Min.	x	x	-
"	60 Min.	-	x	
"	90 Min.	-	x	
"	120 Min.	-	x	
5. No occlusion	30 Min.	x	x	-

ture and to prevent drying of tissues.

Time Studies

Various tissues of the experimental animal were removed following predetermined time intervals. In these studies timing was initiated after injection of the radioisotopic compound and terminated with tissue removal. Thirty minute intervals following acetate ^{3}H and estradiol-17β-^{3}H injections were performed on animals exhibiting surgical variations 3 and 4. A thirty minute study, following acetate-^{3}H injection, was performed on animals subjected to surgical variations 1 and 2. In addition to the 30 minute studies, 2, 6, 10, 15, 60, 90 and 120 minute experiments were performed on animals with the ovarian branches of the ovarian arteries bilaterally occluded. Estradiol-17β-^{3}H was the only isotopic compound used in the 60, 90 and 120 minute experiments while only acetate-^{3}H was used in the 15 minute experiment.

Dianil blue was injected into the femoral vein of each animal in the two minute study and every other animal in the six and ten minute studies after ligation. In these studies there was a delay of five minutes after dianil blue injection before estradiol-17β intrauterine injection. The purpose of the dye injection was to evaluate the effectiveness of the ligations in preventing blood flow to the ovaries. If ligations were incomplete the ovaries rapidly turned blue.

At the termination of each time period, both ovaries and fallopian tubes, the anterior one-third of uterine horns and a portion of the liver were removed from the animal. Each tissue was then weighed

to the nearest 0.1 mg and placed in a bag prepared from dialysis tubing. The bag and tissues were then dried in an oven at 60°C for at least eight hours. In the studies including those animals subjected to bilateral occlusion followed by dianil blue injection, the foregoing procedure was followed but only the ovaries and a portion of the liver were removed from each animal.

Liquid Scintillation Technique

After the tissues were dried, they were prepared for counting radioactive content by the Schöniger technique (1955). The bags were folded gently and placed in individual platinum baskets. A small piece (about 5 x 5 mm.) of black starter paper was inserted between the sac and the basket wall. A one liter Erlenmyer flask was oxygenated for one minute after which time the basket containing the sample was suspended from a combustion head (No. 7 rubber stopper with 3-inch glass hook inserted in the bottom), all of which was rapidly inserted into the neck of the flask as soon as the oxygen tube had been withdrawn. The flask was then placed in a Schöniger igniter (A. H. Thomas Co.) and combustion was initiated by focusing the heat lamp on the black starter paper. When combustion was complete the flask was placed in an insulated can containing dry ice and isopropyl alcohol which froze the tritiated water resulting from the combustion. After freezing for a minimum of 20 minutes, the stopper was loosened and a stream of compressed air was passed into the flask for 10 seconds to remove the excess oxygen remaining after combustion. The flask was then removed from the bath and 17.0 ml of Diotol scintilla-

tion fluid was added. The composition of the Diotol was 30.5 per cent toluene, 35.5 per cent dioxane, 21.5 per cent methanol, 7.5 per cent naphthalene and 5 per cent liquifluor. The flask was then swirled to rinse its sides and allowed to warm to room temperature. Fifteen ml of the Diotol-tritiated water solution were then transferred from the flask into a scintillation sample bottle using a volumetric pipette. Each scintillation vial was placed in a Packard liquid scintillation counter and two 10 minute readings were taken for each sample. Background was checked by measuring the counts per minute (CPM) registered for Diotol fluid alone. In addition, each sample and blank was counted with an internal standard for one minute to determine counting efficiency.

Evaluation of Data

Total CPM were obtained from the average of two 10 minute counting periods per sample on the scintillation counter. Actual disintigrations per minute (DPM) were obtained by subtracting background counts and correcting for counting efficiency. DPM divided by the weight of the tissue in milligrams gave the specific activity of the tissue (DPM/mg wet tissue). In order to determine the percentage of the dose existing in the ovaries and tubules at the end of each study, they were divided by the total amount of injected radioactivity (4.5 μc or 9.99×10^6 DPM).

It was assumed than in order for local transport to have occurred, the radioactive concentration in the ovary ipsilateral to the injected horn must have been consistently greater than the concentration in the

contralateral ovary. The same relationship should have held for the fallopian tubes if local transport between adjacent uterine horns and ovaries traversed the tubes. Values for the ipsilateral and contralateral fallopian tubes were compared without any statistical analysis in all of the studies when tissue counts were apparently the same ($P > .05$). In those studies where tissue counts were not apparently equal, a comparison of sample means of paired observations, as described by Steel and Torrie (1960), was performed. This analysis involved calculating the variance of paired ovaries or paired tubules in each group rather than among the individuals in each group. The student t test was used to determine with what probability the ovaries or tubules being compared were from the same population.

RESULTS AND DISCUSSION

Four surgical procedures (described in the Surgical Procedure section) were used for exploration of local transport. In procedure 1 the anterior and posterior uterine veins were occluded on the same side as the intra-uterine injection. With the venous effluent from the injected uterine horn occluded, the isotopic solutions injected should be restricted from the general circulation. Ideally then, the local transport from the injected uterine horn to its ipsilateral ovary would be favored and systemic levels of radioactivity would remain low for some time. Surgical procedure 2 involved occlusion of the anterior and posterior uterine vein on the side of intra-uterine injection and, in addition, the ligation of arteries associated with the occluded veins. As in procedure 1, the injected radioisotope solution should be restricted from the general circulation. By ligating the arteries, the hydrostatic pressure generated at the uterus should be lower than in procedure 1 because the blood flow was both restricted from entering and leaving the uterine horn. This would give a method of evaluating signs of local transport that might be present as the result of the first procedure.

Isolation of the ovary adjacent to the injected uterine horn was attempted in surgical procedure 3. Isolation was accomplished by occluding the arterial supply to the ovary. It was intended that any radioactivity in the isolated ovary would originate from the local transport system rather than the general circulation. In surgical

procedure 4 an attempt was made to isolate both ovaries from the general circulation. This was done by occluding the arterial affluent to each ovary. This surgical procedure was chosen for the bulk of the work in this investigation because it was apparent from the first two experiments that the radioisotope solution was not being adequately isolated, due to seepage into the general circulation via the cervix branches of the uterine vein and by seepage into the lymph system. With knowledge of this contamination, it was concluded that by subjecting both ovaries to the same surgical isolation and allowing the injected radioisotope to escape freely into the general circulation, a quantitative difference of radioactivity in both ovaries could be more easily detected. In other words, if a direct transport system existed the ovary ipsilateral to the injected uterine horn would have a source of radioactive input while the contralateral ovary would not. Also blocking the arterial supply should have the effect of decreasing background levels, which would allow smaller differences to be more obvious. Initially groups of animals were studied when no surgical alterations were made on the circulatory system associated with the uterine horns or ovaries. The radioisotope levels measured from these animals should serve as an indication of the normal levels of isotope in all the tissues following intra-uterine injection. A comparison of the radioactive levels in the tissues of these animals and the tissues in the animals subjected to vascular ligations should give an indication of the relative amount of isotope moving to the tissues after the ligations.

Those tissues assayed for tritium content in the time studies

with intervals equal to or greater than 15 minutes after the radioisotopic solution was injected were both ovaries, both fallopian tubes, the upper one-third of both uterine horns and a portion of the liver. The injected uterine horn was studied because the radioactivity remaining in this tissue at the end of the time study would be an indication of the amount of radioisotope that had migrated from the horn and, as it was the recipient of the total injection, its radioactive count should serve as a reference for accuracy of the injection. The non-treated horn was selected primarily to determine if contamination occurred due to spillage of the radioisotope, contamination by blood or migration of the isotope from one uterine horn to the other via the cervices. Animals exhibiting radioactivity in this horn which approach the activity of the injected horn or were substantially greater than the activity in the liver were considered to be contaminated and discarded.

Although information from the literature indicates that local transport between adjacent uterine horns and ovaries via the fallopian tube is not the route of the luteolytic factor (Kiracofe et al., 1963; Deanesly, 1967), both fallopian tubes were examined for radioactivity in an attempt to evaluate if local communication between a uterine horn and ovary could follow this pathway. If the tubule on the side injected contained substantially greater amounts of radioactivity than the tubule on the non-injected side, possible transport via the fallopian tube would be indicated.

The liver was examined for radioactive content because its concentration would reflect the general level of radioactivity in the

circulation. It also is a tissue, similar to the tubules, ovary and uterine horns, that readily utilizes acetate and estradiol-17β. Finally the liver acts as a source for determining the level of the radioisotope in the general circulation. Large radioactive concentration in the liver and the non-injected uterine horn implies high vascular levels.

The information obtained from examination of the ovaries was the exclusive information used in determining whether a local transport system exists. Consistently greater radioactive concentrations in the ovaries adjacent to the injected uterine horns than found in the ovaries contralateral to the injected horn would imply the presence of a local transport system.

It should be pointed out that the accumulation of the radioactivity in the tissues depends on both the relative concentration of the radioisotope in the blood and the tissues and the relative affinity of the radioisotope for the blood and tissues (Jensen and Jacobson, 1962). This situation is obviously confused by metabolism of the radioactive substance and some accumulation of end products, and these errors would probably increase with the length of the study.

Surgical Experiment 1

The purpose of Surgical Experiment 1 was to isolate the radioisotopic material injected into one uterine horn from the general circulation and then measure the concentration of radioactivity in both ovaries, both fallopian tubes, the upper one-third of both uterine horns and a portion of the liver. A 30 minute study was

performed using tritiated acetate injected into the uterine lumen.

Results for this study are outlined in Table 2. Because of consistently higher counts (DPM/mg wet tissue) for the ipsilateral tubules and ovaries in comparison to the contralateral tubules and ovaries, a statistical evaluation was done. Tabulations for this study indicated no significant difference in the radioactive content of the tissues ($.20>P>.10$) for both the two ovaries and the two tubules.

In retaining the criterion that the differences between the ipsilateral and contralateral ovary must be significant ($P<.05$), this part of the experiment does not support the presence of a local transport system between ipsilaterally located uterine horn and ovary. Furthermore, because there was no difference between the radioactive concentration in the ipsilateral and contralateral ovaries or ipsilateral and contralateral tubules, a transport via the fallopian tube was not demonstrated with this study.

The values for the ovaries and tubules in animals 4 and 5 deviate from values of the other animals in the group. The results indicate a greater concentration of radioisotope on the ipsilateral side than on the contralateral side. This observation led the investigator to believe that the volume of injection was so great that the isotope solution was forced not only into the uterine lumen but also into the lumen of the tubule and maybe onto the ovary located ipsilateral to the injected horn. Therefore, although a direct transport occurred, it was not considered to represent a physiological situation.

Table 2: Radioactivity 30 minutes after acetate-^{3}H injection into guinea pigs with unilateral occlusion of the uterine veins. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	1	2	3	4
Tissue				
Ovary**				
Contralateral	0.2(0.4)*	2.9(7.9)	1.5(6.6)	3.9(8.1)
Ipsilateral	0.4(1.0)	2.9(7.5)	2.1(8.6)	37.1(60.1)
Tubule**				
Contralateral	8.7(5.8)	1.0(2.7)	0.9(1.7)	7.2(10.5)
Ipsilateral	0.8(1.0)	2.4(5.6)	17.8(34.3)	355.7(1096.7)
Uterus				
Contralateral	2.7	1.9	23.1	8.5
Injected	734.6	234.0	1485.0	2,211.0
Liver	0.6	1.3	1.1	1.8

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

** $\underline{t}$ = $P > .05$

Table 2: Continued

Animal Number	5	6	7	8
Tissue				
Ovary**				
Contralateral	9.2(17.2)	3.0(11.5)	2.0(9.8)	0.9(2.6)
Ipsilateral	85.1 (149.0)	4.2(15.1)	2.1(14.5)	1.4(4.5)
Tubule**				
Contralateral	6.9(19.4)	2.9(0.7)	1.7(8.2)	1.0(3.1)
Ipsilateral	247.8(441.4)	29.1(44.9)	1.3(6.2)	0.7(2.0)
Uterus				
Contralateral	7.0	457.3	0.7	0.9
Injected	575.9	529.2	179.0	14.8
Liver	5.2	2.5	2.0	0.7

Surgical Experiment 2

The purpose of Surgical Experiment 2 was the same as described in Surgical Experiment 1 but the arteries associated with the uterine veins were also ligated (see Table 1). A 30 minute study using tritiated acetate was performed.

Results for this study are outlined in Table 3. As in the first study discussed (uterine vein occlusion), a statistical evaluation was made on the difference values between the ipsilateral and contralateral ovaries. The evaluations indicated no significant difference between counts in the ipsilateral and contralateral ovaries ($.40 > P > .20$). No statistical analysis was made on the differences between fallopian tubes because it was apparent that the radioactivity in both tissues was the same. This part of the experiment suggested a lack of a local transport system and also suggested a lack of local transport via the fallopian tube.

When the median values for the levels of activity (DPM/mg wet tissue) of tissues between Tables 2 and 3 were emperically compared (Table 19), it was found that the median values were consistently greater for all the tissue in Table 3. It was the thought of this investigator that the opposite of these observed results should have been obtained because the arterial supply to the uterus probably produced greater than normal hydrostatic pressure in the study represented in Table 2, while lower hydrostatic pressure should have been present at the uterine horns in the study represented by Table 3. The increased hydrostatic pressure would exert an effect on the

Table 3: Radioactivity 30 minutes after acetate-^{3}H injection into guinea pigs with unilateral occlusion of the uterine veins and associated arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	9	10	11
Tissue			
Ovary**			
Contralateral	6.1(11.0)*	11.8(20.3)	5.9(12.6)
Ipsilateral	8.0(13.7)	12.2(25.8)	7.0(15.8)
Tubule			
Contralateral	4.9(10.9)	80.1(156.3)	5.7(6.7)
Ipsilateral	15.4(37.7)	20.3(22.9)	5.4(6.4)
Uterus			
Contralateral	598.0	511.0	177.0
Injected	626.0	2341.0	318.0
Liver	4.4	8.7	2.7

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

** $\underline{t}$ = P>.05

uterus to increase transudate formation favoring an increased lymphatic drainage from the uterus thus increasing radioisotope drainage to the general circulation. Another possible explanation for these observations may simply be in the amount of animals used in each study. Perhaps the three animals used for evaluation in Table 3 were not representative of the population and therefore led to incorrect observations. Analysis of the counts in the contralateral uterine horn of the animals in Table 3 indicated high radioactive concentration. This possible contamination may have served as an additional supply of radioisotope to the general circulation which would indeed account for higher activity in the other tissues of the body.

Surgical Experiment 3

The purpose of Surgical Experiment 3 was to isolate the ovary, ipsilateral to the injected uterine horn, from the general circulation and then measure the level of radioactivity in the same tissues described in the two previous studies. Isolation of the ipsilateral ovary was performed as described in Table 1. Thirty minute studies, using tritiated acetate and estradiol-17β, were performed.

Results for the acetate-^{3}H study are outlined in Table 4 and for the estradiol-17β study in Table 5. An emperical evaluation was made in both studies for the differences in radioactivity between ipsilateral and contralateral tissues.

The results indicated no difference in radioactive concentrations in the two ovaries and also no difference in the levels of radioactivity in the two fallopian tubes. No conclusive information was

obtained from this part of the experiment to indicate the presence or absence of a local transport system. In this study equal counts in both ovaries might demonstrate local transport because it has not been determined what the difference in radioactive counts between ovaries must be to indicate local transport. Since similar radioactive concentration of the two ovaries was detected, local transport could be present.

It should be noted that in the first two studies the radioisotope was isolated as completely as possible from the general circulation. In this study only one ovary was selectively isolated from the general circulation. Since it was not determined what level of isotope in the isolated ovary would represent local transport this method was further modified. The next set of experiments was attempted to estimate relative levels of radioactivity in the two ovaries when both were isolated from the general circulation.

Surgical Experiment 4

In Surgical Experiment 4 (Table 1) the radioactive concentration in the same tissues was analyzed as in the previous studies. Exception to this procedure were those studies conducted for 2, 6 and 10 minutes where only the ovaries and a portion of the liver were analyzed for radioactivity. An additional step was included in these studies. This was to inject intravenously 1 ml of dye, dianil blue (1%), immediately after occluding the blood vessels. A 5 minute period was allowed after the dye injection to permit the dye to be completely circulated. In all the animals subjected to the dye, a blue coloration

Table 4: Radioactivity 30 minutes after acetate-^{3}H injection into guinea pigs with unilateral occlusion of the ovarian branches of the ovarian artery. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	12	13	14	15	16	17	18
Tissue							
Ovary							
Contralateral	5.8(22.8)*	1.2(4.5)	4.3(11.2)	1.7(7.8)	4.2(15.2)	3.1(12.7)	0.9(2.5)
Ipsilateral	4.0(14.1)	1.2(3.6)	3.6(9.9)	1.1(3.9)	3.8(14.4)	3.1(11.8)	1.2(2.5)
Tubule							
Contralateral	4.8(21.2)	1.8(3.3)	2.9(5.5)	1.5(2.2)	2.0(6.4)	2.3(6.4)	1.0(2.7)
Ipsilateral	8.3(33.8)	4.0(3.4)	3.8(9.8)	0.9(2.3)	12.4(32.9)	3.1(5.4)	2.3(3.3)
Uterus							
Contralateral	199.0	3.1	391.0	2.5	219.0	2.7	2.2
Injected	516.6	154.0	785.0	185.5	762.0	712.0	166.0
Liver	2.8	1.0	1.8	1.0	2.5	2.0	0.3

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

Table 5: Radioactivity 30 minutes after estradiol-17β-^{3}H injection into guinea pigs with unilateral occlusion of the ovarian branches of the ovarian artery. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	19	20	21	22	23	24
Tissue						
Ovary						
Contralateral	0.7(2.4)*	2.2(6.8)	2.3(4.0)	2.1(4.0)	1.3(4.5)	3.5(13.7)
Ipsilateral	1.9(3.4)	1.9(5.5)	1.8(3.5)	1.2(2.5)	0.0(0.0)	2.6(10.3)
Tubule						
Contralateral	1.6(2.8)	2.2(5.5)	2.2(7.4)	1.4(3.5)	2.1(5.7)	3.9(3.4)
Ipsilateral	1.7(7.1)	3.4(8.8)	3.2(13.2)	2.8(11.7)	2.2(6.1)	4.1(14.8)
Uterus						
Contralateral	0.7	2.8	2.5	2.3	1.3	3.0
Injected	274.5	302.0	114.7	72.4	244.7	128.4
Liver	1.5	2.6	6.0	5.4	2.0	6.8

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

appeared in all the organs of the body except the ovaries which had their arterial supply ligated. This demonstrated complete occlusion of the arterial supply to the ovaries. Studies using estradiol-17β injected into the uterine lumen were made at 120, 90, 60, 30, 10, 6 and 2 minute durations between isotope injection and tissue removal. Studies were also made using acetate injection for 30 and 15 minute intervals.

Results for these studies are outlined in Tables 6-14. Emperical evaluations were made for the studies conducted for 120, 90, 60, 30, 10 and 6 minute durations. In these studies it was apparent that there was no difference between the radioactivity in the ipsilateral and contralateral ovaries and no difference between the radioactive concentrations in ipsilateral and contralateral tubules. The studies performed at 15 and 2 minutes were analyzed with the statistical method used previously. It was found that in both studies no significant difference could be demonstrated for the radioactive levels in the two ovaries ($.40>P>.20$ for the 15 minute study; $.20>P>.10$ for the 2 minute study). This part of the research also does not demonstrate the presence of a local transport system or transport via the fallopian tube.

Again as in Surgical Experiment 1, large counts in ipsilateral ovaries and tubules in animals 38, 52, 59, 69, 75 and 76 possibly indicate errors in the injection process. It is the belief of this investigator that the isotope was injected into both the uterine and tubule lumen and also onto the surface of the ovary leading to the high radioactivity in these animals.

Table 6: Radioactivity 120 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/wet tissue).

Animal Number	25	26	27	28	29
Tissue					
Ovary					
Contralateral	1.2(2.9)*	0.7(4.3)	1.4(5.8)	1.7(2.8)	1.1(3.7)
Ipsilateral	1.5(5.6)	1.1(6.7)	1.7(4.7)	1.3(4.0)	0.8(2.9)
Tubule					
Contralateral	1.9(4.8)	1.6(7.1)	4.1(15.9)	5.7(3.7)	1.1(4.1)
Ipsilateral	2.8(16.1)	2.7(11.0)	2.3(10.3)	2.5(7.5)	1.2(5.8)
Uterus					
Contralateral	2.0	1.9	4.8	6.4	1.9
Injected	67.7	14.1	35.1	46.1	31.1
Liver	1.2	2.1	1.6	2.0	2.5

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 120 minutes after radioisotope injection.

Table 7: Radioactivity 90 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	30	31	32	33	34
Tissue					
Ovary					
Contralateral	1.4(4.3)*	1.6(3.3)	1.8(6.2)	2.3(8.3)	3.6(11.0)
Ipsilateral	1.4(3.9)	1.2(2.7)	2.4(9.1)	1.7(6.6)	3.5(10.2)
Tubule					
Contralateral	2.2(4.9)	2.6(2.0)	2.4(8.7)	4.3(21.9)	5.2(10.8)
Ipsilateral	2.9(13.5)	1.6(5.1)	2.6(12.9)	3.2(7.7)	5.4(11.3)
Uterus					
Contralateral	1.8	1.8	130.2	37.2	4.2
Injected	95.5	38.7	472.7	132.0	153.0
Liver	2.9	1.5	5.2	2.0	5.0

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 90 minutes after radioisotope injection.

Table 8: Radioactivity 60 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	35	36	37	38	39	40
Tissue						
Ovary						
Contralateral	0.5(2.6)*	1.2(5.4)	0.5(1.9)	2.0(4.1)	0.4(1.6)	0.9(1.4)
Ipsilateral	0.3(1.5)	1.5(5.9)	0.4(1.3)	7.1(9.4)	1.2(4.8)	0.7(1.7)
Tubule						
Contralateral	0.8(4.5)	2.2(7.5)	1.4(1.2)	2.0(9.9)	1.2(2.8)	1.6(2.2)
Ipsilateral	1.0(3.6)	3.7(9.9)	0.5(2.0)	91.0(339.8)	3.3(5.4)	0.9(3.7)
Uterus						
Contralateral	0.8	5.3	1.5	1.4	0.7	1.3
Injected	641.0	92.1	5.1	504.0	395.0	44.1
Liver	0.8	3.5	0.3	2.8	1.3	1.0

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 60 minutes after radioisotope injection.

Table 9: Radioactivity 30 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	41	42	43	44	45	46
Tissue						
Ovary						
Contralateral	0.1(0.2)*	1.8(6.6)	1.0(4.5)	2.0(6.0)	1.2(4.0)	2.6(15.1)
Ipsilateral	0.2(0.7)	2.1(6.1)	1.6(3.7)	- (-)**	1.1(2.8)	2.6(12.1)
Tubule						
Contralateral	0.0(0.0)	1.1(2.6)	0.8(1.3)	6.1(13.0)	1.9(2.9)	5.3(8.4)
Ipsilateral	0.3(0.7)	2.4(8.6)	2.6(6.0)	4.1(7.6)	2.0(4.7)	4.2(17.3)
Uterus						
Contralateral	0.1	38.3	0.8	7.2	1.6	3.0
Injected	2.1	116.0	455.5	48.8	142.2	80.6
Liver	-**	3.2	-**	7.2	2.0	4.5

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

** Tissues lost in process of scintillation preparation.

Table 10: Radioactivity 30 minutes after acetate-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of net tissue (DPM/mg wet tissue).

Animal Number	47	48	49	50	51	52
Tissue						
Ovary						
Contralateral	4.2(13.4)*	0.8(3.6)	1.5(7.2)	3.2(8.1)	2.7(9.3)	2.7(7.7)
Ipsilateral	2.2(6.3)	1.0(3.9)	2.3(12.4)	2.5(6.4)	0.7(2.6)	7.0(13.6)
Tubule						
Contralateral	2.6(27.7)	1.5(4.5)	2.2(3.8)	2.3(7.3)	3.1(10.4)	5.2(14.6)
Ipsilateral	2.8(8.0)	1.4(4.1)	5.0(8.6)	3.7(10.5)	1.5(7.6)	4.6(16.1)
Uterus						
Contralateral	1.3	1.0	0.9	1.5	4.7	-**
Injected	4706.0	298.0	1452.0	115.4	127.7	300.3
Liver	1.7	1.0	1.0	1.5	1.6	2.1

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

** Tissues lost in process of scintillation preparation.

Table 11: Radioactivity 15 minutes after acetate-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values and disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	53	54	55	56
Tissue				
Ovary***				
Contralateral	1.0(4.4)*	0.5(1.5)	6.2(24.7)	3.2(12.0)
Ipsilateral	1.0(3.8)	0.3(0.7)	6.6(26.9)	4.2(7.4)
Tubule				
Contralateral	18.7(24.1)	1.4(2.9)	8.7(18.9)	5.4(5.9)
Ipsilateral	6.7(23.1)	1.5(5.8)	5.2(24.7)	5.6(6.9)
Uterus				
Contralateral	52.9	1.2	2.2	76.1
Injected	595.3	-**	77.3	318.9
Liver	8.5	0.7	1.6	1.6

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 15 minutes after radioisotope injection.

** Tissue lost in process of scintillation preparation.

*** $\underline{t}$ = $P>.05$

Table 11: Continued

Animal Number	57	58	59	60
Tissue				
Ovary***				
Contralateral	0.8(2.7)	0.5(3.0)	2.4(10.3)	0.8(1.7)
Ipsilateral	1.6(3.2)	0.6(2.9)	133.4(363.3)	1.2(1.8)
Tubule				
Contralateral	1.6(2.9)	0.8(3.0)	2.4(7.9)	0.9(2.6)
Ipsilateral	1.5(4.1)	1.0(6.2)	639.5(2125.3)	1.1(2.9)
Uterus				
Contralateral	139.0	120.0	202.4	0.7
Injected	120.5	226.0	308.1	119.6
Liver	1.1	0.7	2.0	0.7

Table 12: Radioactivity 10 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	61	62	63	64	65
Tissue					
Ovary					
Contralateral	0.2(0.8)*	0.5(2.3)	0.3(1.2)	0.7(4.0)	1.4(1.9)
Ipsilateral	0.5(2.0)	0.3(1.8)	0.5(2.2)	0.4(2.7)	2.8(8.3)
Liver	0.4	0.4	2.4	1.6	2.0

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 10 minutes after radioisotope injection.

Table 13: Radioactivity 6 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	66	67	68	69	70
Tissue					
Ovary					
Contralateral	0.7(2.6)*	0.7(1.8)	0.9(2.1)	0.5(1.0)	0.5(3.6)
Ipsilateral	0.2(0.6)	0.2(0.9)	0.0(0.0)	4.9(7.4)	0.4(8.3)
Liver	0.2	0.2	1.0	0.3	0.7

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 6 minutes after radioisotope injection.

Table 14: Radioactivity 2 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	71	72	73	74
Tissue				
Ovary				
Contralateral	0.1(0.4)*	0.1(0.2)	0.1(0.2)	0.0(0.0)
Ipsilateral	0.1(0.4)	0.1(0.4)	0.2(0.5)	0.7(1.9)
Liver	0.0	0.0	0.1	0.1

Animal Number	75	76	77	78
Tissue				
Ovary				
Contralateral	0.1(0.4)	0.3(1.3)	0.3(1.4)	0.5(1.5)
Ipsilateral	7.9(20.7)	2.6(10.6)	0.5(1.8)	0.4(1.0)
Liver	0.0	0.4	0.5	0.3

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 2 minutes after radioisotope injection.

** $\underline{t}$ = P>.05

GENERAL DISCUSSION

Initially a study was done to determine the levels of radioactivity in tissues when no surgical variations of the circulatory system had been performed. The purpose of this study was to obtain data which could be used as an indication of normal levels of activity in tissues after uterine injections of tritiated estradiol-17β or acetate. It has been well documented (Jensen, 1965; Flescher, 1965) that labled steroids injected intravenously and subcutaneously have been found in high concentrations in the uterine myometrium and especially in the uterine endometrium, as well as other tissues of the body. Time studies which showed levels of activity in tissues after intra-uterine injection of estradiol have also been made (Stone and Martin, 1964), but the tissues examined for radioactive concentration in this study differed from the earlier study. Therefore two 30 minute studies, one using acetate-^{3}H and the other estradiol-17β-^{3}H, were performed with this procedure. The results for these two studies are summarized in Tables 15 and 16. The median values in DPM/mg wet tissue analyzed for radioactive content was also determined for these studies (Table 17). Median values were also calculated for the estradiol-17β studies (Table 18), and the acetate studies (Table 19).

In comparing the estradiol-17β experiments, it was observed that slightly larger amounts of the radioactive substance had migrated to the ovaries in the animals without alteration of the vascular system than those animals subjected to ovarian vascular isolation. Consider-

ably lesser amounts of the isotope were detected in the 2, 6 and 10 minute time studies with bilateral ovarian isolation than the amount detected for the 30 minute study involving bilateral ovarian isolation. A slight drop in radioactive content was then observed in the tissues at 60 minutes after injection which then increased to the 30 minute activity level at 90 minutes after injection and again decreased slightly in the 120 minute study. The activity levels in the unilaterally isolated ovaries performed at 30 minutes after injection were greater than the activity levels in ovaries subjected to bilateral isolation. However the activity in the ipsilateral ovaries, which were isolated in both studies, differed by only 0.3 DPM/mg wet tissue.

When 0.11 μg (12.8 μc) of tritiated estradiol were injected subcutaneously in seasame oil into mice (Stone, 1963; Stone, Baggett and Donelly, 1963), absorption of the hormone was gradual and blood levels of radioactivity remained constant for several hours. Similar responses were observed in the "non-target" tissues, i.e. the kidney, adrenal, lung, skeletal muscle, bone, hypothalmus, cerebrum, liver and the ovary. On the other hand the uterus, vagina and anterior pituitary exhibited continuous uptake for several hours so that the peak of radioisotope concentration occurred 6 hours after injection. If the estradiol was given subcutaneously in saline solution, absorption was rapid; the blood levels of radioactivity reached a maximum within about 15 minutes after which it steadily decreased. Again the patterns of radioactivity in the "non-target" tissues paralleled that of blood, but in the "target" tissues (uterus, vagina and pituitary),

Table 15: Radioactivity 30 minutes after estradiol-17β-^{3}H injection into guinea pigs. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	79	80	81
Tissue			
Ovary			
Contralateral	3.6(13.3)*	2.8(11.3)	1.7(3.3)
Ipsilateral	11.3(38.8)	2.7(8.1)	1.1(3.3)
Tubule			
Contralateral	5.1(21.2)	2.8(4.2)	1.5(4.5)
Ipsilateral	16.3(4317)	3.5(3.8)	1.2(3.5)
Uterus			
Contralateral	21.0	2.1	1.0
Injected	61.7	148.6	12.3
Liver	9.0	3.4	3.1

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

Table 16: Radioactivity 30 minutes after acetate-^{3}H injection into guinea pigs. Values are disintigrations per minute per milligram of wet tissue (DPM/mg wet tissue).

Animal Number	82	83	
Tissue			
Ovary			
Contralateral	1.9(6.4)*	2.5(11.3)	2.8(5.8)
Ipsilateral	1.7(3.9)	1.6(6.4)	3.4(5.7)
Tubule			
Contralateral	1.8(5.1)	1.2(4.0)	4.7(7.5)
Ipsilateral	1.1(3.6)	1.6(4.0)	4.3(10.5)
Uterus			
Contralateral	0.8	2.6	-**
Injected	5.4	14.9	3.9
Liver	0.6	0.6	59.3

* Values in parenthesis represent the per cent ($x10^{-6}$) of injected radioisotope present in the entire tissue 30 minutes after radioisotope injection.

** Tissue lost in process of scintillation preparation.

Table 17: Median values for radioisotope concentration (DPM/mg wet tissue) in tissues when no surgical variation of the circulatory system was performed before isotope injection. The values in column 1 represent the estradiol study and column 2 represents the acetate study.

Time*	Estradiol 30 Minutes	Acetate 30 Minutes
Tissue		
Ovary		
Contralateral	2.8	2.5
Ipsilateral	2.7	1.7
Tubule		
Contralateral	2.8	1.8
Ipsilateral	3.5	1.6
Uterus		
Contralateral	2.1	1.7
Injected	61.7	5.4
Liver	3.4	0.6

* Time between injection of radioisotope solution and tissue removal.

Table 18: Median values for radioisotope concentration (DPM/mg wet tissue) in tissues with either unilateral occlusion (UO) or bilateral occlusion (BO) of the ovarian branches of the ovarian arteries prior to estradiol-17β-^{3}H injection.

Time Surgery	120 Min. BO	90 Min. BO	60 Min. BO	30 Min. BO	10 Min. BO	6 Min. BO	2 Min. BO	30 Min. UO
Tissue								
Ovary								
Contralateral	1.1	1.8	0.7	1.5	0.5	0.7	0.1	2.1
Ipsilateral	1.3	1.7	1.0	1.6	0.5	0.2	0.5	1.9
Tubule								
Contralateral	1.9	2.6	1.8	1.4				2.2
Ipsilateral	2.5	2.9	2.1	2.5				3.0
Uterus								
Contralateral	2.0	4.2	1.4	3.4				2.4
Injected	35.1	132.0	200.0	100.0				180.0
Liver	2.0	2.9	1.2	3.9	1.6	0.3	0.1	3.9

* Time between injection of estradiol and tissue removal.

Table 19: Median values for radioisotope concentration (DPM/mg wet tissue) in tissues with either unilateral occlusion (UO) or bilateral occlusion (BO) of the ovarian branches of the ovarian artery and unilateral occlusion of the venous effluent (UV) or unilateral occlusion of the venous effluent and associated arteries (UVA) of the injected uterine horn prior to acetate-^{3}H injection.

Time* / Surgery	30 Min. UV	30 Min. UVA	30 Min. UO	30 Min. BO	15 Min. BO
Tissue					
Ovary					
Contralateral	2.5	6.1	3.1	2.7	0.9
Ipsilateral	2.5	8.0	3.1	2.3	1.4
Tubule					
Contralateral	2.3	5.7	2.0	2.5	2.0
Ipsilateral	10.0	15.4	3.8	3.3	3.3
Uterus					
Contralateral	4.9	511.1	3.1	1.3	27.2
Injected	555.0	626.0	516.6	299.0	226.0
Liver	1.5	4.4	1.8	1.6	1.4

* Time between injection of acetate-^{3}H and tissue removal.

there is a prolonged uptake and retention of the radioactive steroid (Jensen, DeSombre, Hurst, Kawashima and Jungblut, unpublished). In the present study, it appeared that the liver had attained its peak activity by 30 minutes after intra-uterine injection and decreased to half this value by 120 minutes after injection. The ovary, on the other hand, seemed to be more in line with the aforementioned experiments in which, once a radioactive level was achieved, that level was maintained. Although the actual time for reaching this plateau was not determined, it is apparent that it was reached before 30 minutes after the injection.

A peculiar feature of the studies using estradiol-17β-^{3}H as the radioactive tracer was that the levels of radioactivity in the ovaries remained nearly constant from 30 minutes to 120 minutes after injection. Considering the studies of Stone (1963) and Stone et al., (1963), these results were expected if the ovaries had a constant source of the material. It has already been pointed out that the ovaries probably received the radioisotope from the general circulation in those experiments in which the uterine veins or the uterine veins and associated arteries were occluded prior to radioisotope injection. In these studies the radioisotope probably escaped via the cervix veins. When the arterial supply to the ovaries was occluded, it was initially presumed that the ovaries were isolated from the general circulation. Evaluating the radioactive levels in the ovary after this surgery, led this investigator to conclude that isolation of the ovary from the general circulation was not complete. The source of radioactive contamination probably originated from the bursa

(which was counted with the ovary) and tubule which were not restricted from receiving radioisotope from the general circulation. Since the flow to the tubule was probably constant the radioactive levels in the ovary probably reflect this flow and would be constant.

In evaluating the acetate studies for radioactive levels, the first thing that was apparent was that larger amounts of isotope were incorporated in the ovaries than with the estradiol studies. Plateau values of radioisotope uptake appears to occur before 30 minutes and possibly before 15 minutes after injection in the ovaries and liver. This probably indicates faster uptake of the acetate than estradiol. A striking feature of the acetate study, was that when the injected radioisotope was isolated from the general circulation, higher counts were found in all the tissues analyzed when compared to the counts in tissues when no isolation was performed. It would be of great interest to pursue these interesting observations with other studies to see if variations of radioactivity levels occur with time.

The research could also be improved by conducting time studies of less than two minutes when both ovaries are isolated from the general circulation. It must be pointed out that this study was limited because of the rapid uptake of the radioisotope and because of the fast circulation time for rodents. Therefore, lower counts would be observed in tissues if the studies were performed for times less than two minutes than those observed for time studies greater than two minutes. If differences between radioactive levels in tissues exists, they would be more readily apparent. In performing the various occlusions of the blood vessels, it might be beneficial in future studies

to sever as well as to ligate them. This alteration would eliminate any seepage of the radioisotope through the ligation. When both ovaries are isolated from the general circulation, it also might be beneficial to occlude the vessels leading to the tubules to help prevent flow of radioisotope to the ovary via this tissue. The volume of the injection should also be reduced to prevent the possibility of abnormal distension or trauma to the uterine horn. Finally, future studies might include radioisotopic compounds other than acetate-^{3}H and estradiol-17β-^{3}H dissolved in saline. An attempt would thus be made to mimic the normal condition of local transport if it exists.

SUMMARY

The radioactive levels of the ipsilateral and contralateral ovaries were statistically equal in all the studies performed in this research. In those studies in which both ovaries were isolated from the general circulation or when the injected uterine horn was isolated from the general circulation, the statistical equality of the ipsilateral and contralateral ovaries demonstrated a lack of local uteroovarian transport. In those studies in which the ovary ipsilateral to the injected horn was isolated from the general circulation, the equal levels of radioactivity in both the ipsilateral and contralateral ovaries neither supported nor refuted the presence of a local uteroovarian transport because the difference in radioactive levels between the ipsilateral and contralateral ovaries which would have indicated local transport was not defined.

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APPENDIX A

Total DPM in Tissues Analyzed

Table 1: Total DPM 30 minutes after acetate-^{3}H injection into guinea pigs with unilateral occlusion of the uterine vein.

Animal Number	1	2	3	4	5	6	7	8
Tissue								
Ovary								
Contralateral	4	79	66	80.6	171.4	114.8	98.0	25.7
Ipsilateral	10	75	86	600.4	1,488	150.6	141.4	44.7
Tubule								
Contralateral	58	27	17	104.8	193.4	448.0	82.3	30.7
Ipsilateral	10	56	343	10,956	4,410	74.5	62.3	20.2
Uterus								
Contralateral	446	305	2,525	718.3	776.9	41,019	713.6	134.7
Injected	104,756	46,579	173,740	298,514	76,591	61,066	54,013	3,574
Liver	163	146	271	449.2	974.2	670.6	359.8	206.4

Table 2: Total DPM 30 minutes after acetate-^{3}H injection into guinea pigs with unilateral occlusion of the uterine veins and associated arteries

Animal Number	9	10	11
Tissue			
Ovary			
Contralateral	109.6	257.7	125.6
Ipsilateral	136.6	203.0	157.4
Tubule			
Contralateral	108.8	1,561.0	66.8
Ipsilateral	376.8	229.0	63.7
Uterus			
Contralateral	50,188.0	63,946.0	16,925.0
Injected	63,509.0	287,781.0	27,762.0
Liver	1,231.0	1,072.0	469.3

Table 3: Total DPM 30 minutes after acetate-^{3}H injection into guinea pigs with unilateral occlusions of the ovarian branches of the ovarian artery.

Animal Number	12	13	14	15	16	17	18
Tissue							
Ovary							
Contralateral	228.0	44.7	112.1	78.1	151.9	127.0	24.8
Ipsilateral	141.0	36.2	98.8	38.7	143.9	117.9	24.8
Tubule							
Contralateral	212.0	33.1	55.1	21.9	63.8	63.8	27.0
Ipsilateral	338.0	33.6	98.3	22.9	328.3	53.8	32.5
Uterus							
Contralateral	18,368.0	265.1	32,060.0	287.3	31,103.0	192.2	183.4
Injected	80,894.0	8,250.0	53,026.0	26,492.0	74,081.0	59,703.0	11,427.0
Liver	561.0	229.9	548.7	292.7	858.5	356.1	102.9

Table 4: Total DPM 30 minutes after estradiol-17β-^{3}H injection into guinea pigs with unilateral occlusion of the ovarian branches of the ovarian artery.

Animal Number	19	20	21	22	23	24
Tissue						
Ovary						
Contralateral	24.4	68.2	40.0	39.5	44.5	136.4
Ipsilateral	33.8	54.4	35.2	24.8	00.0	102.7
Tubule						
Contralateral	27.8	54.4	74.1	34.5	57.0	33.9
Ipsilateral	71.1	88.0	131.8	117.2	60.7	147.7
Uterus						
Contralateral	228.5	300.1	263.4	388.0	170.5	715.9
Injected	68,480.0	23,295.0	15,856.0	12,142.0	28,560.0	7,881.0
Liver	296.5	586.9	1,749.0	2,085.0	758.8	1,079.0

Table 5: Total DPM 120 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	25	26	27	28	29
Tissue					
Ovary					
Contralateral	28.5	43.1	58.3	27.5	37.0
Ipsilateral	55.6	67.2	46.7	40.2	28.6
Tubule					
Contralateral	45.8	70.4	158.8	37.3	41.0
Ipsilateral	160.4	110.0	102.4	74.8	58.2
Uterus					
Contralateral	340.6	495.0	746.6	931.1	174.8
Injected	13,497.0	2,846.0	6,734.0	6,670.0	1,952.0
Liver	201.5	239.0	323.9	206.3	610.5

Table 6: Total DPM 90 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	30	31	32	33	34
Tissue					
Ovary					
Contralateral	43.2	33.2	62.0	83.0	109.5
Ipsilateral	39.0	27.0	90.7	65.5	101.7
Tubule					
Contralateral	49.4	20.3	86.7	218.5	107.8
Ipsilateral	134.6	51.3	128.7	77.1	113.1
Uterus					
Contralateral	302.8	233.0	22,581.0	5,537.0	940.7
Injected	22,576.0	6,141.0	16,450.0	14,693.0	28,966.0
Liver	476.2	295.3	943.9	305.4	859.1

Table 7: Total DPM 60 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	35	36	37	38	39	40
Tissue						
Ovary						
Contralateral	25.9	54.4	18.8	41.3	15.7	13.8
Ipsilateral	14.5	58.7	13.3	94.2	47.7	17.2
Tubule						
Contralateral	44.5	75.3	11.9	98.8	28.1	22.4
Ipsilateral	35.7	98.5	19.9	3,395.0	54.4	37.0
Uterus						
Contralateral	89.5	1,231.0	327.2	221.4	100.3	21.1
Injected	57,046.0	14,369.0	806.4	114,115.0	58,929.0	11,671.0
Liver	142.5	748.2	60.5	361.8	187.4	130.1

Table 8: Total DPM 30 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	41	42	43	44	45	46
Tissue						
Ovary						
Contralateral	2.0	66.3	45.0	59.9	40.2	150.4
Ipsilateral	7.0	60.4	37.3	-*	27.5	121.0
Tubule						
Contralateral	0.0	25.7	13.0	130.2	28.5	84.3
Ipsilateral	7.0	86.2	59.8	76.3	46.7	172.3
Uterus						
Contralateral	30.0	17,942.0	154.1	465.6	434.1	436.9
Injected	499.0	40,874.0	118,934.0	6,272.0	23,916.0	9,316.0
Liver	-*	458.0	-*	2,464.0	557.2	899.8

* Tissues lost in process of scintillation preparation.

Table 9: Total DPM 30 minutes after acetate-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	47	48	49	50	51	52
Tissue						
Ovary						
Contralateral	133.8	35.5	72.3	80.8	93.2	76.9
Ipsilateral	63.2	39.3	124.1	64.3	25.8	136.0
Tubule						
Contralateral	39.4	44.5	37.9	72.5	103.4	146.1
Ipsilateral	79.8	41.1	85.4	104.5	76.2	160.6
Uterus						
Contralateral	245.7	136.4	189.9	185.2	406.0	-*
Injected	94,732.0	33,923.0	200,416.0	15,523.0	15,404.0	52,115.0
Liver	276.4	258.7	238.8	397.1	427.7	486.0

* Tissue lost in process of scintillation preparation.

Table 10: Total DPM 15 minutes after acetate-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	53	54	55	56	57	58	59	60
Tissue								
Ovary								
Contralateral	44.0	15.0	247.0	119.9	26.5	29.6	102.5	17.1
Ipsilateral	38.0	7.0	269.0	74.0	32.2	28.9	3,629.0	18.4
Tubule								
Contralateral	240.0	29.0	189.0	58.8	29.1	29.9	78.6	25.9
Ipsilateral	230.0	58.0	247.0	68.4	40.5	61.5	21,232.0	29.2
Uterus								
Contralateral	9,108.0	171.0	353.0	25,043.0	19,998.0	20,225.0	16,877.0	140.1
Injected	74,169.0	-*	15,507.0	86,462.0	20,690.0	35,704.0	33,426.0	15,231.0
Liver	1,517.0	208.0	415.0	344.2	107.3	170.5	398.3	121.0

* Tissue lost in process of scintillation preparation.

Table 11: Total DPM 10 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	61	62	63	64	65
Tissue					
Ovary					
Contralateral	8.4	23.4	12.3	40.3	19.2
Ipsilateral	20.3	17.6	22.1	26.8	82.9
Liver	72.5	45.8	191.6	176.4	330.5

Table 12: Total DPM 6 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	66	67	68	69	70
Tissue					
Ovary					
Contralateral	25.7	17.7	21.2	10.0	35.5
Ipsilateral	5.5	9.3	0.4	74.4	32.8
Liver	44.5	2.6	95.3	37.1	143.8

Table 13: Total DPM 2 minutes after estradiol-17β-^{3}H injection into guinea pigs with bilateral occlusion of the ovarian branches of the ovarian arteries.

Animal Number	71	72	73	74	75	76	77	78
Tissue								
Ovary								
Contralateral	4.4	1.9	1.6	0.0	4.0	12.7	14.3	15.4
Ipsilateral	3.7	3.8	5.1	18.6	207.2	105.4	17.6	9.6
Liver	1.5	6.3	17.8	7.4	3.2	28.4	14.4	12.1

Table 14: Total DPM 30 minutes after estradiol-17β-^{3}H injection into guinea pigs.

Animal Number	79	80	81
Tissue			
Ovary			
Contralateral	131.7	111.8	32.1
Ipsilateral	383.9	80.3	32.5
Tubule			
Contralateral	209.9	41.3	44.5
Ipsilateral	432.4	38.1	34.2
Uterus			
Contralateral	3,299.1	256.9	218.8
Injected	7,566.2	28,763.0	3,412.9
Liver	1,488.8	570.9	402.2

Table 15: Total DPM 30 minutes after acetate-^{3}H injection into guinea pigs.

Animal Number	82	83	84
Tissue			
Ovary			
Contralateral	63.2	111.8	57.7
Ipsilateral	38.6	63.4	56.2
Tubule			
Contralateral	50.7	36.9	73.9
Ipsilateral	35.7	39.2	104.1
Uterus			
Contralateral	132.0	390.5	293.7
Injected	956.5	2,608.8	4,129.6
Liver	169.5	211.5	-*

* Tissue lost in process of scintillation preparation.